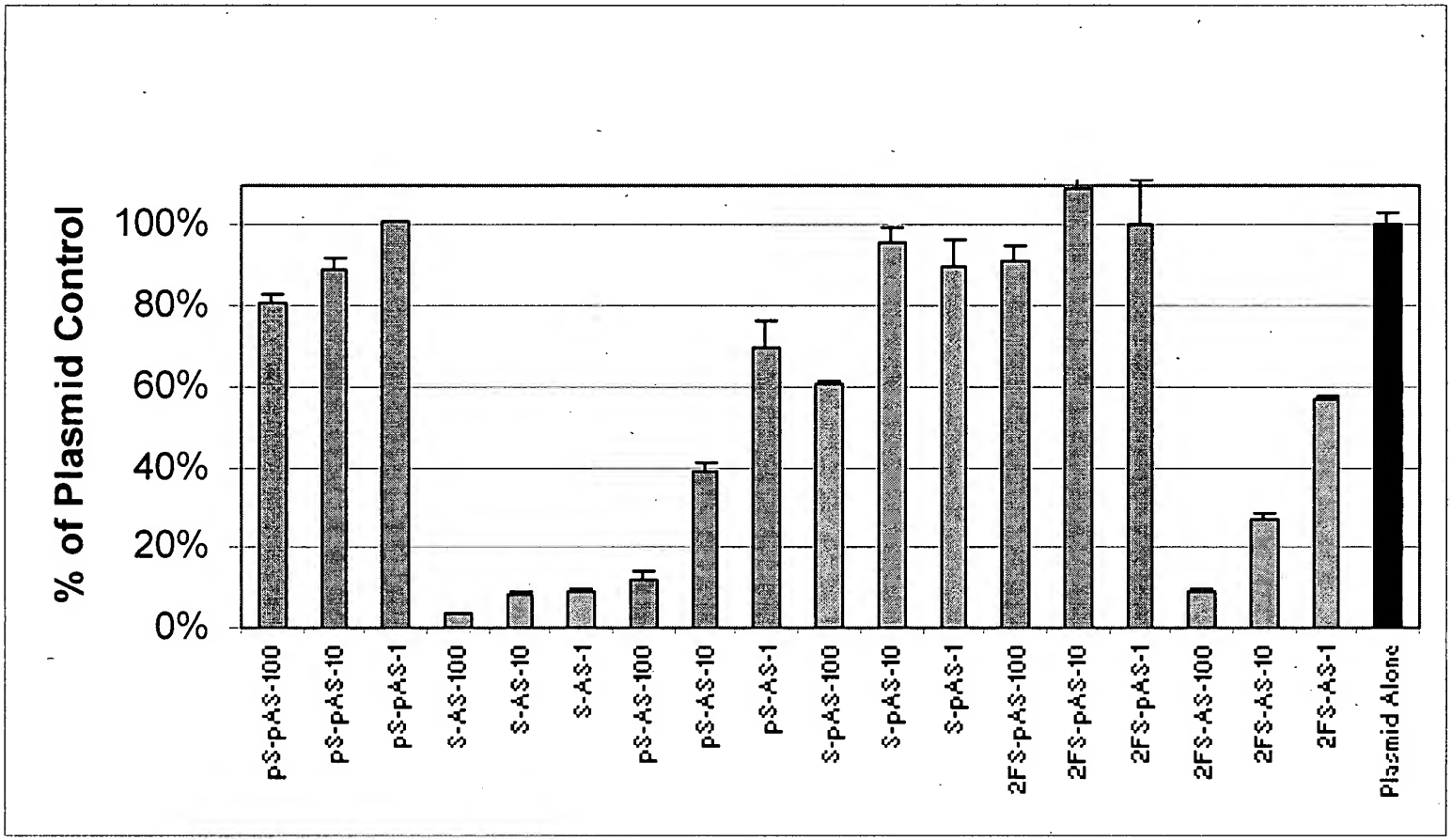



**Figure 1A. Introduction of orthoester modifications to the sense strand of siRNA duplex results in a functional entity, 24 hour time point.**

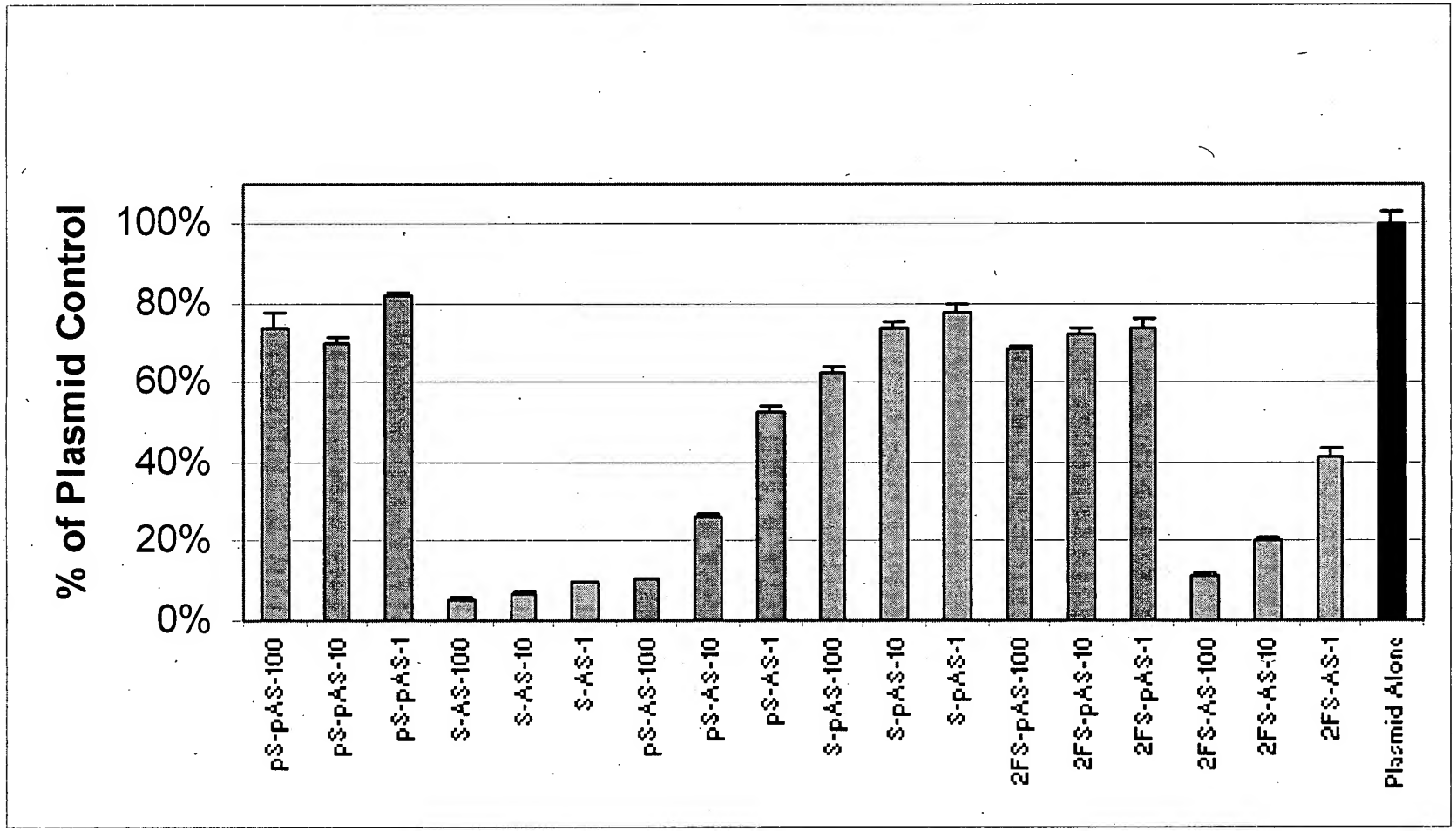



**Figure 1B. Introduction of orthoester modifications to the sense strand of an siRNA duplex results in a functional entity, 48 hour time point.**

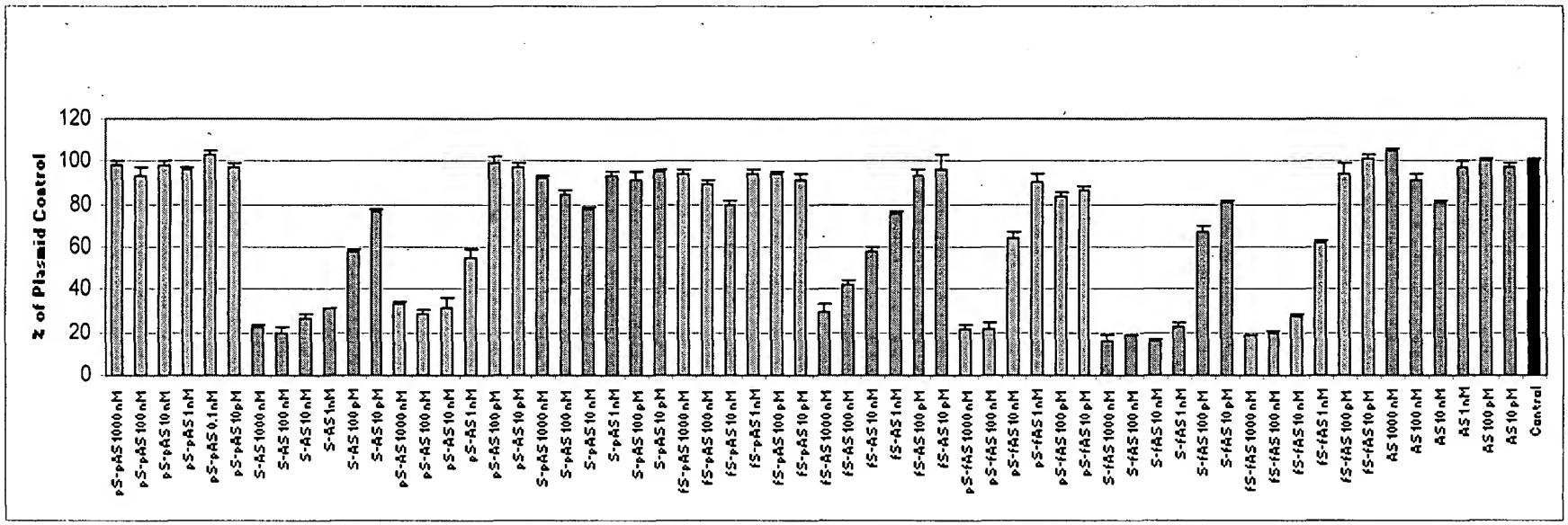



**Figure 2A. Time course of orthoester and 2'F modified siRNAs in cell culture, 24 hour time point.**

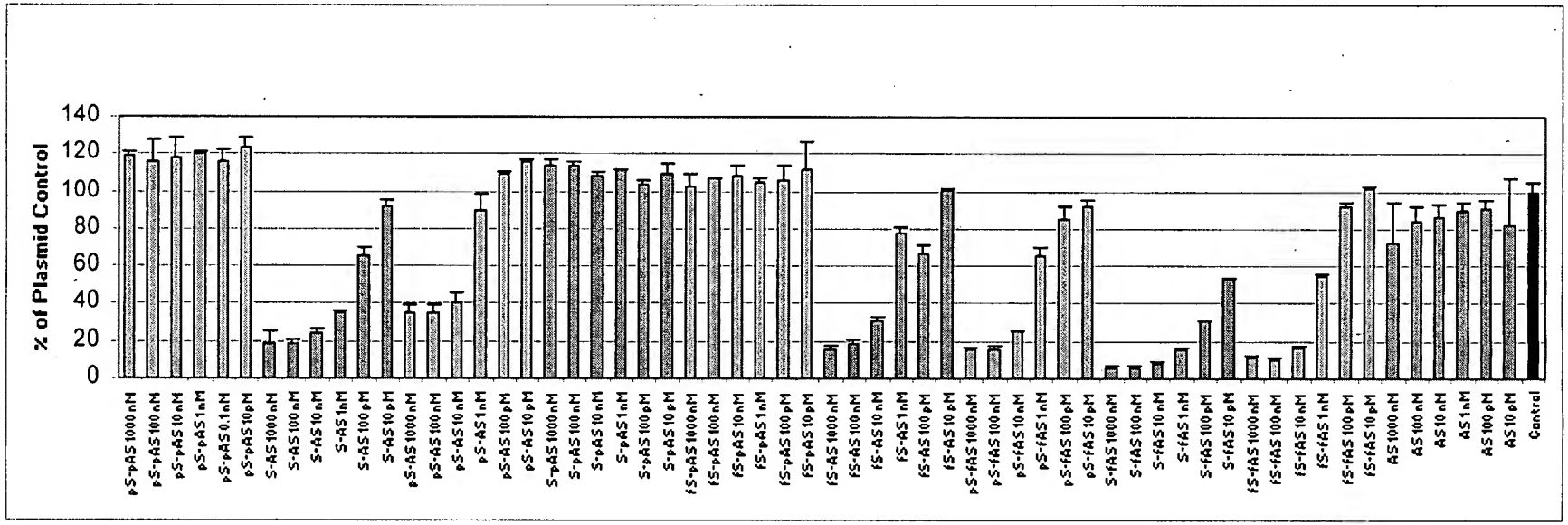



**Figure 2B. Time course of orthoester and 2'F modified siRNAs in cell culture, 72 hour time point.**

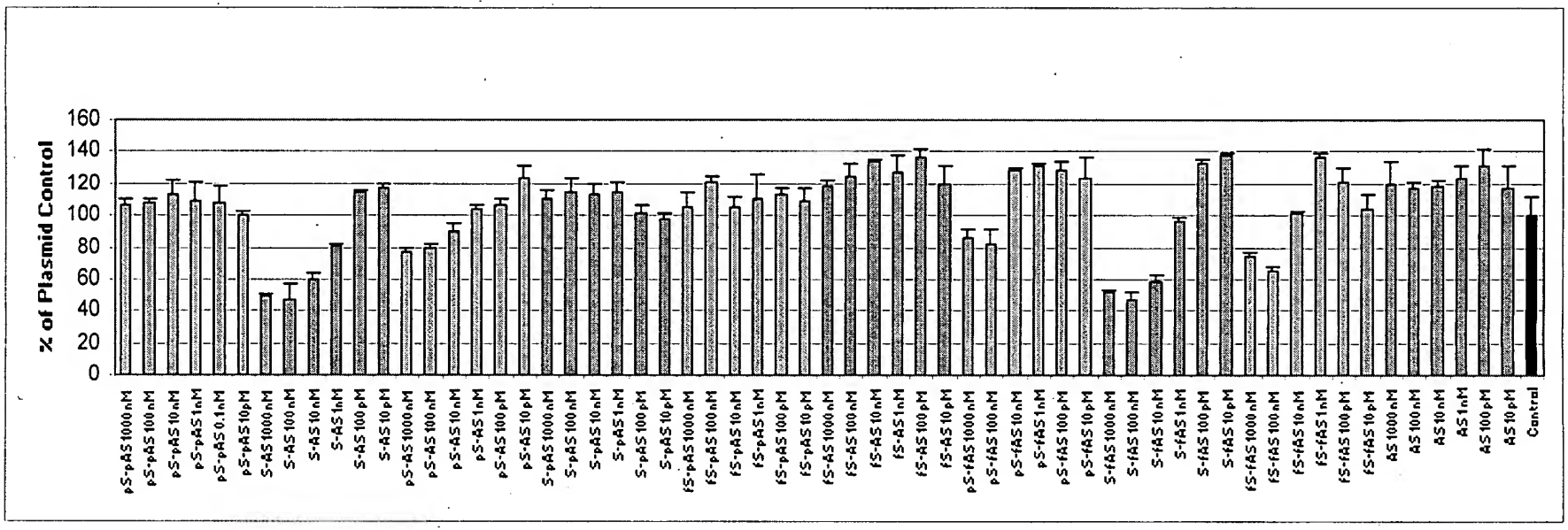



**Figure 2C. Time course of orthoester and 2'F modified siRNAs in cell culture, 144 hour time point.**

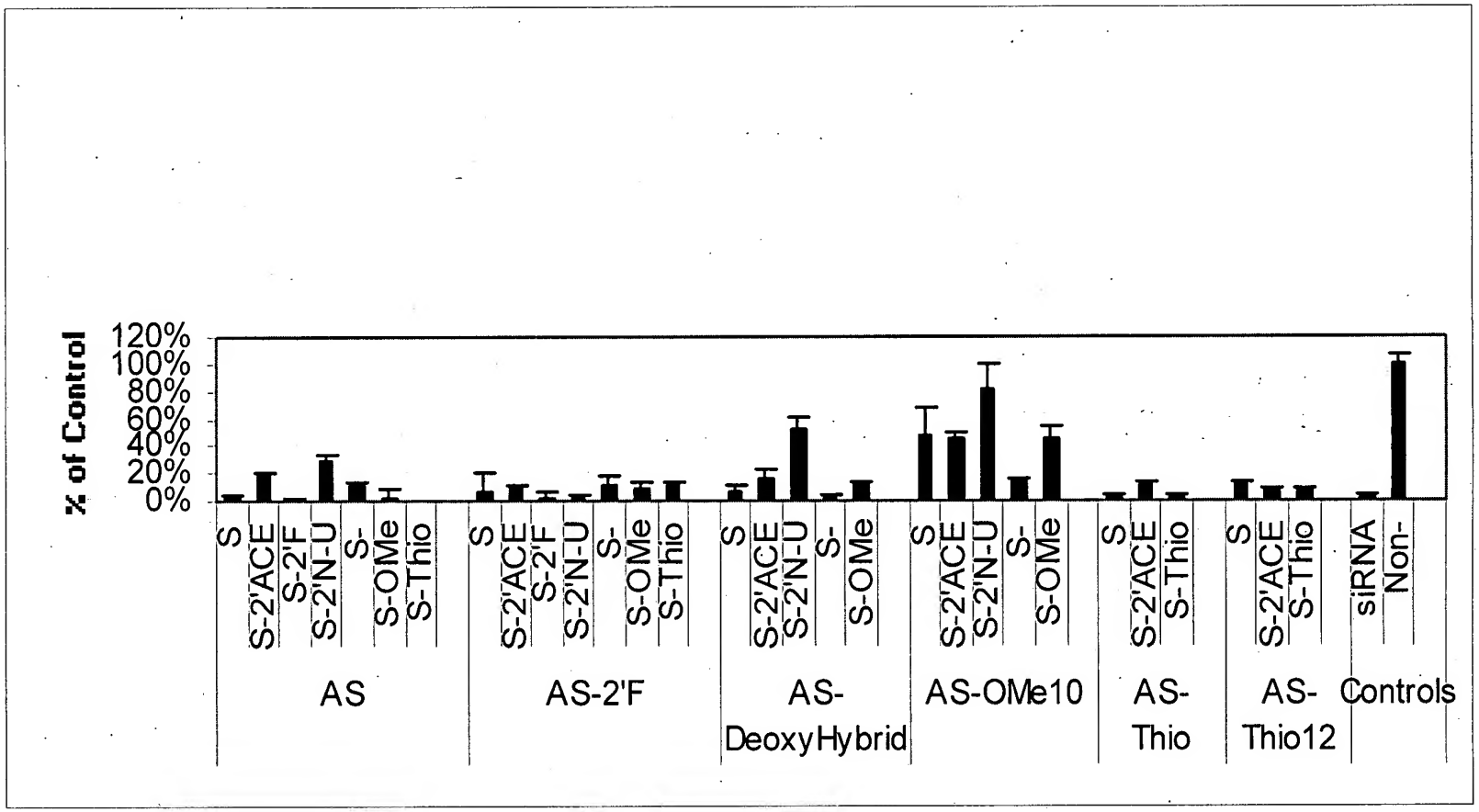



**Figure 3. Modifications tolerance in siRNA: sense screen.**

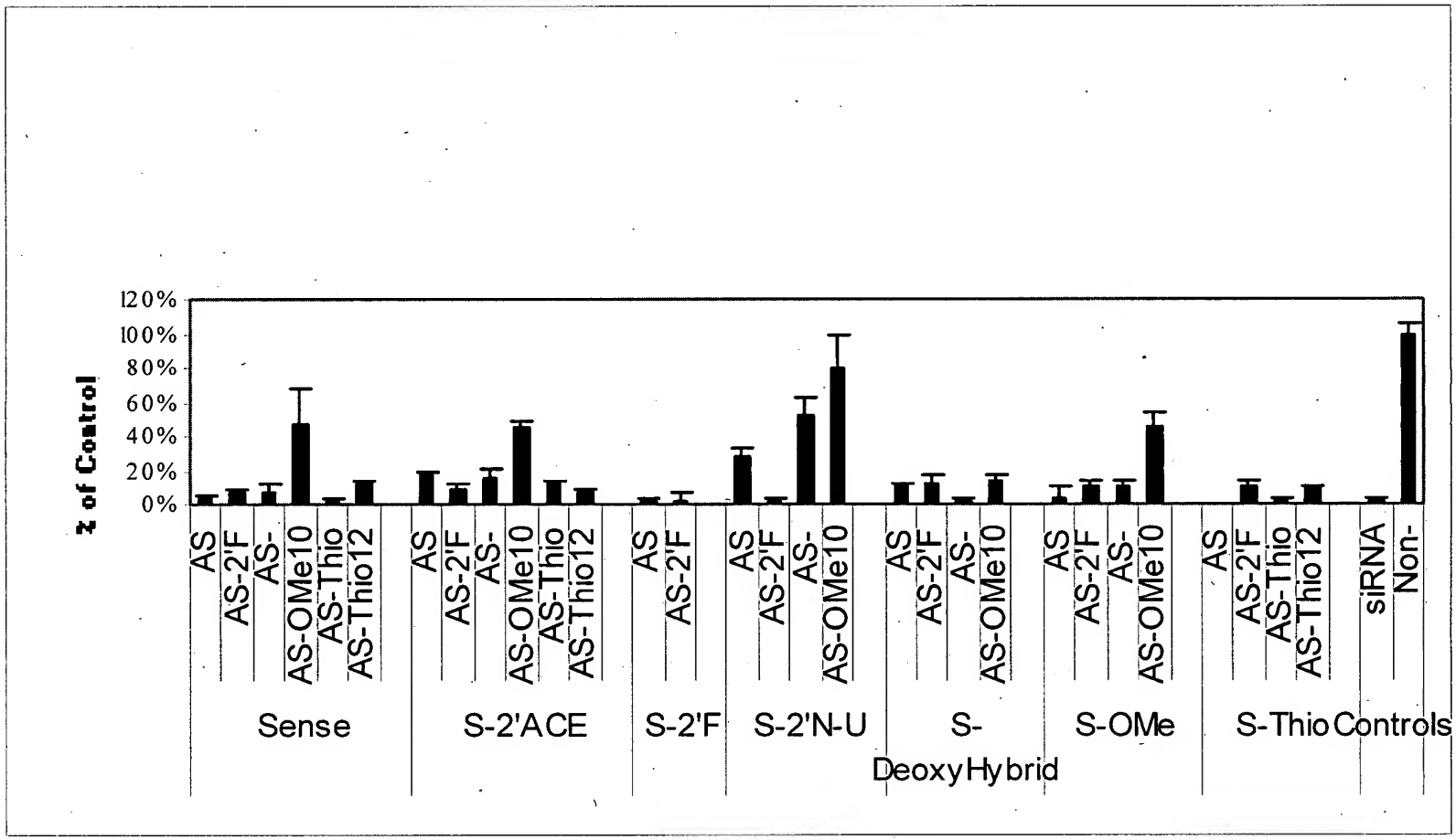



**Figure 4. Modifications tolerance in siRNA: antisense screen.**

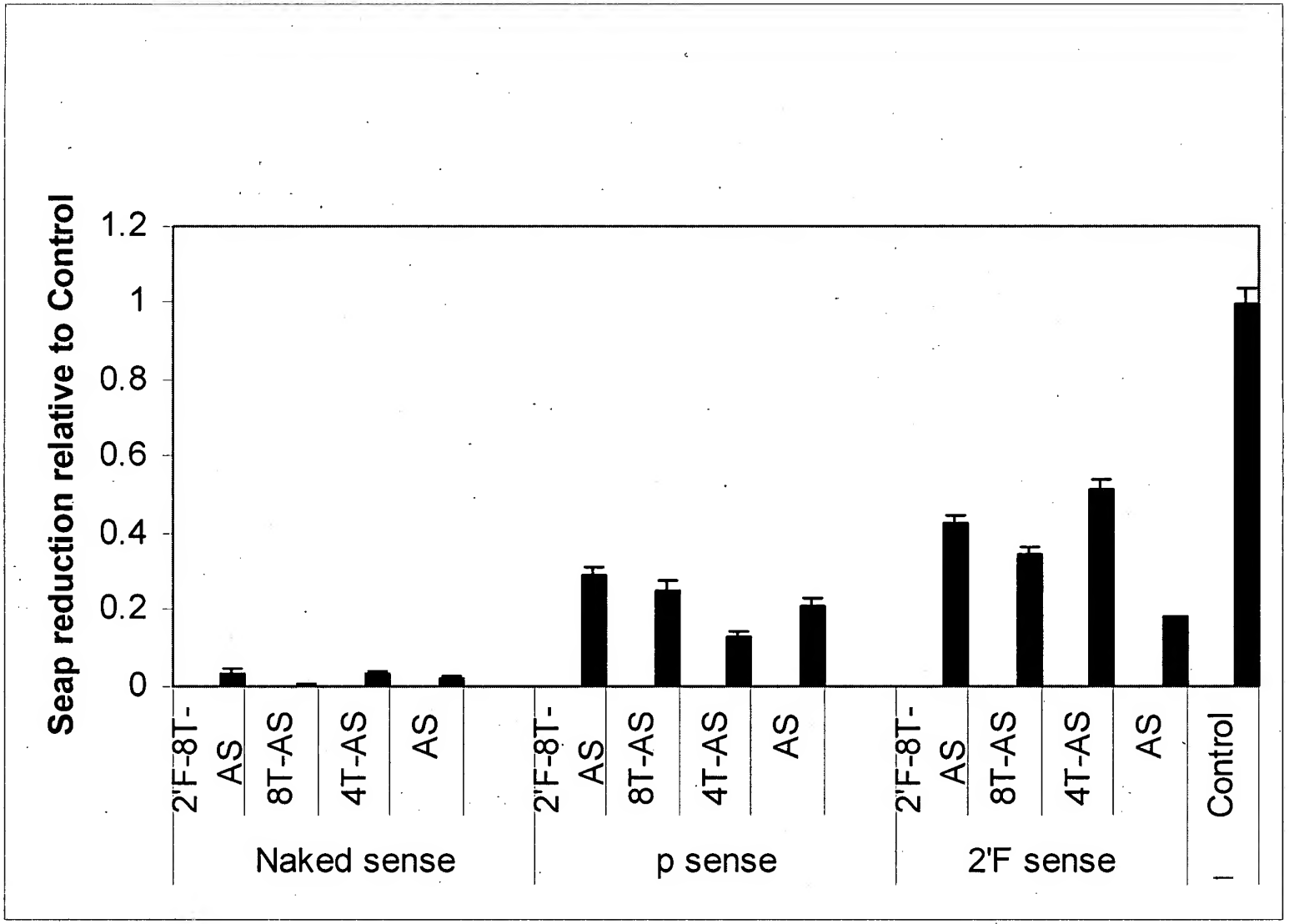



**Figure 5. Thio-Based Modifications on the antisense strand**

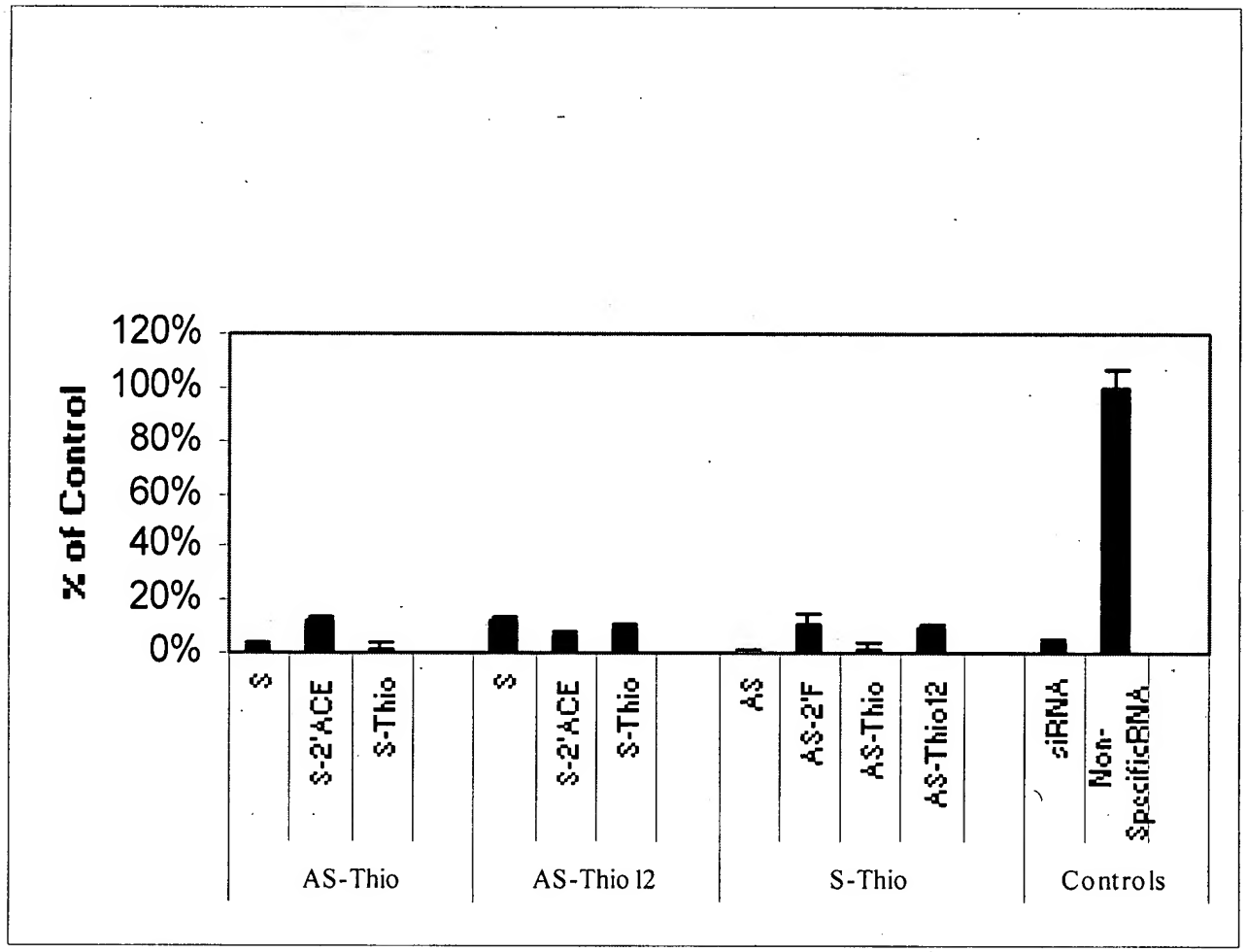



**Figure 6. Phosphorothioate modifications are tolerable in both sense and antisense strands**

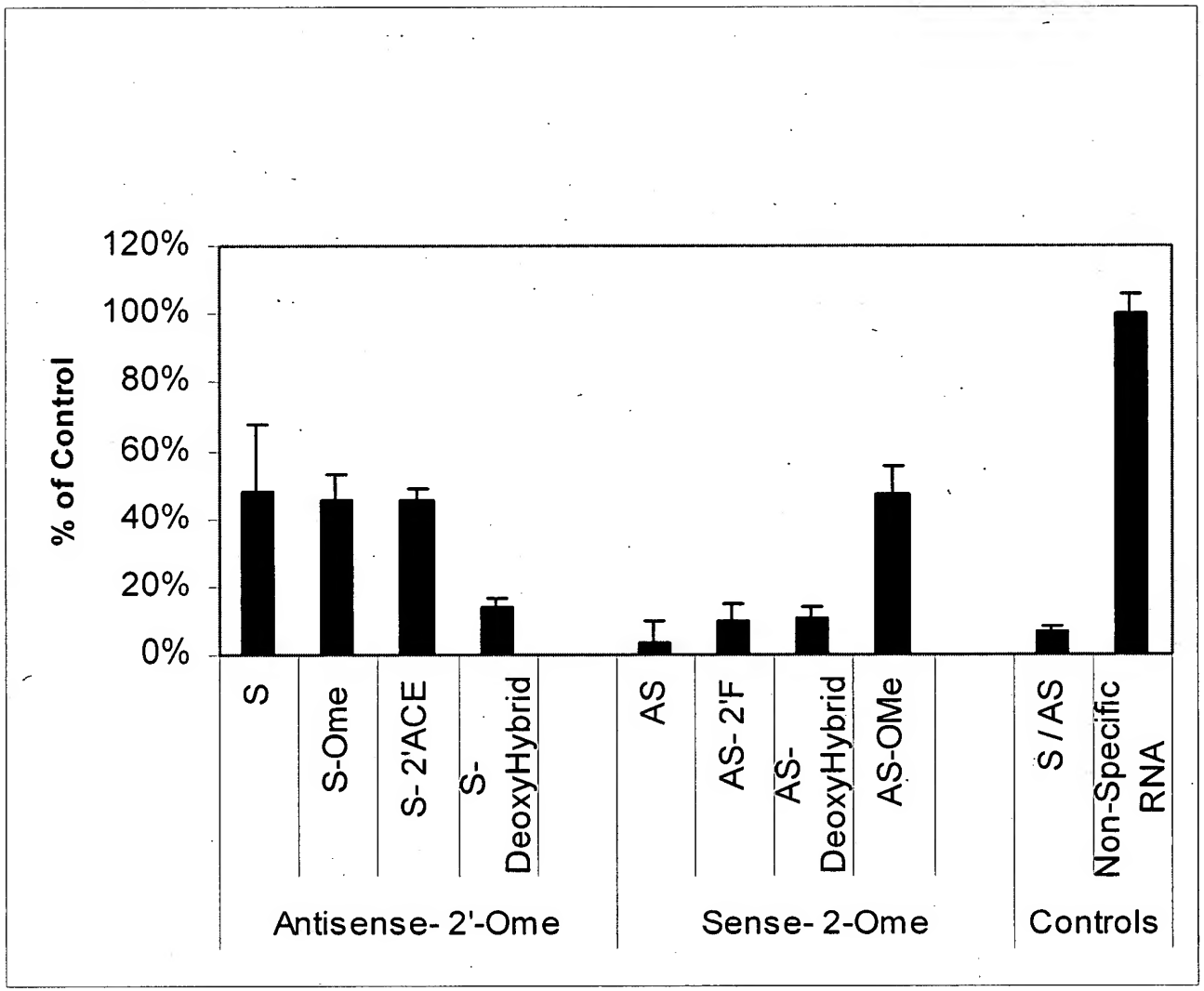



**Figure 7. 2'-O-Methyl modifications in RNA interference.**

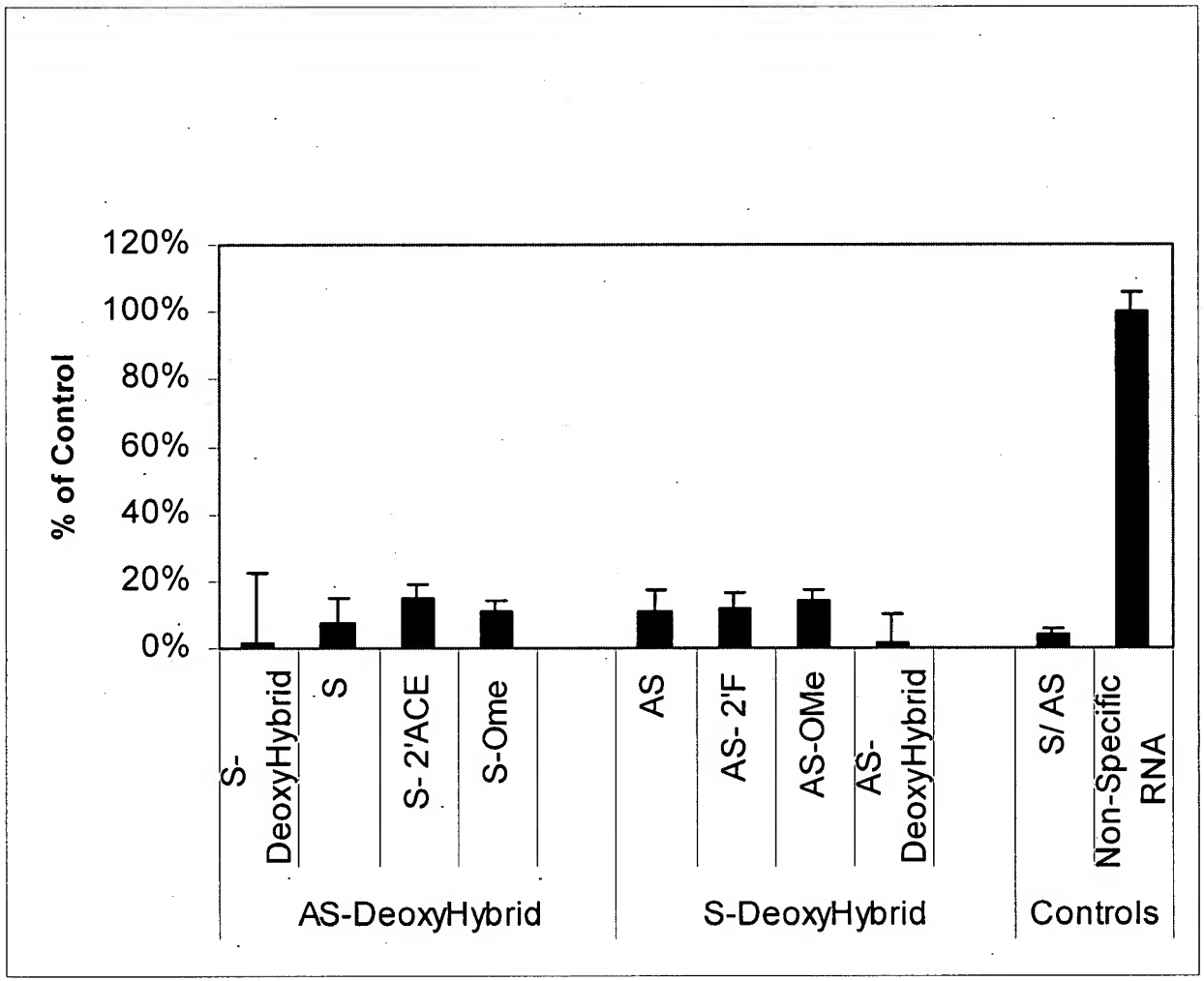



**Figure 8. 2'Deoxy-ribo hybrids in RNA intereference**

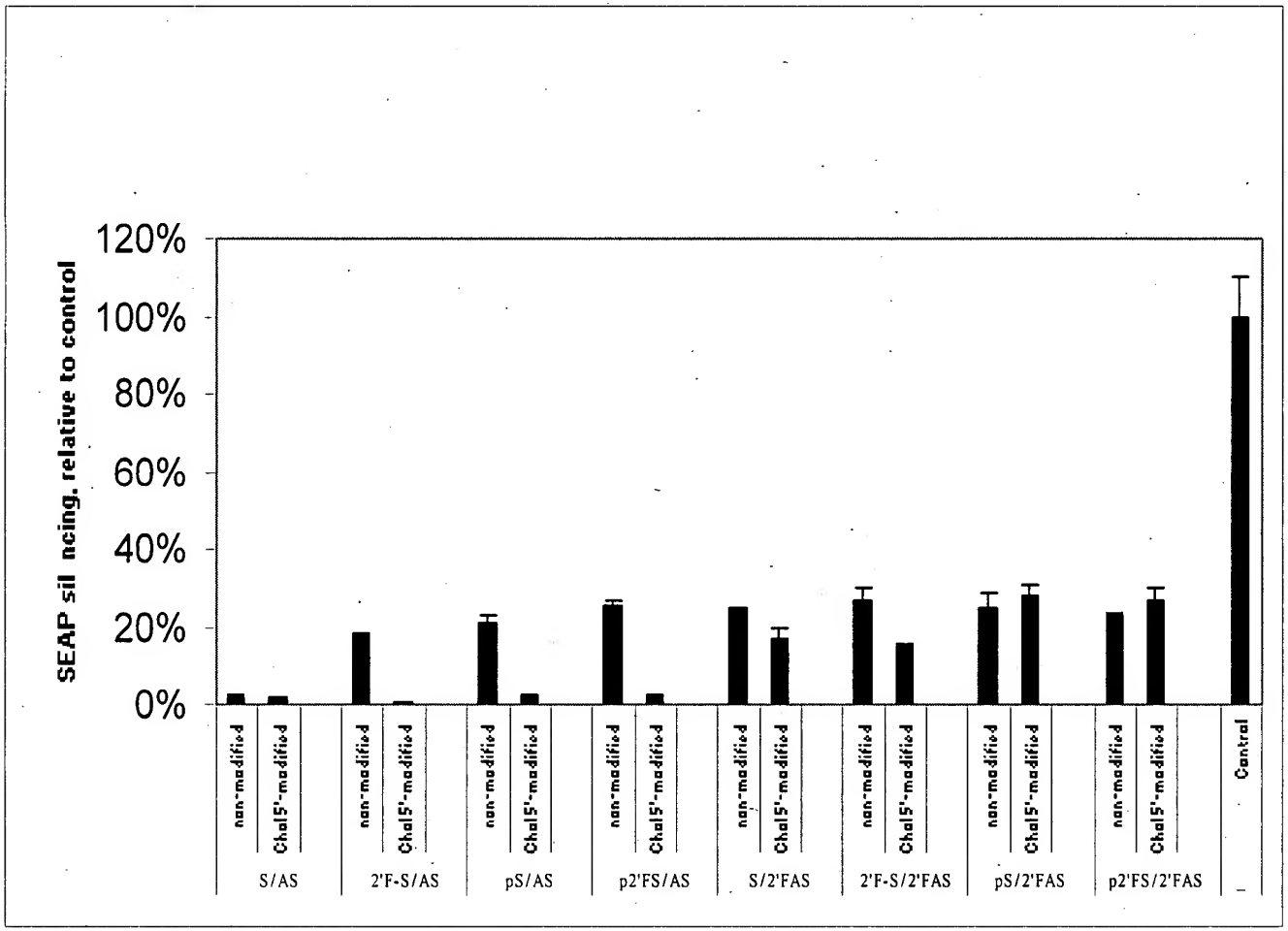



**Figure 9. A cholesterol conjugate on the 5' end of the sense strand of an siRNA duplex increases potency of modified siRNA**

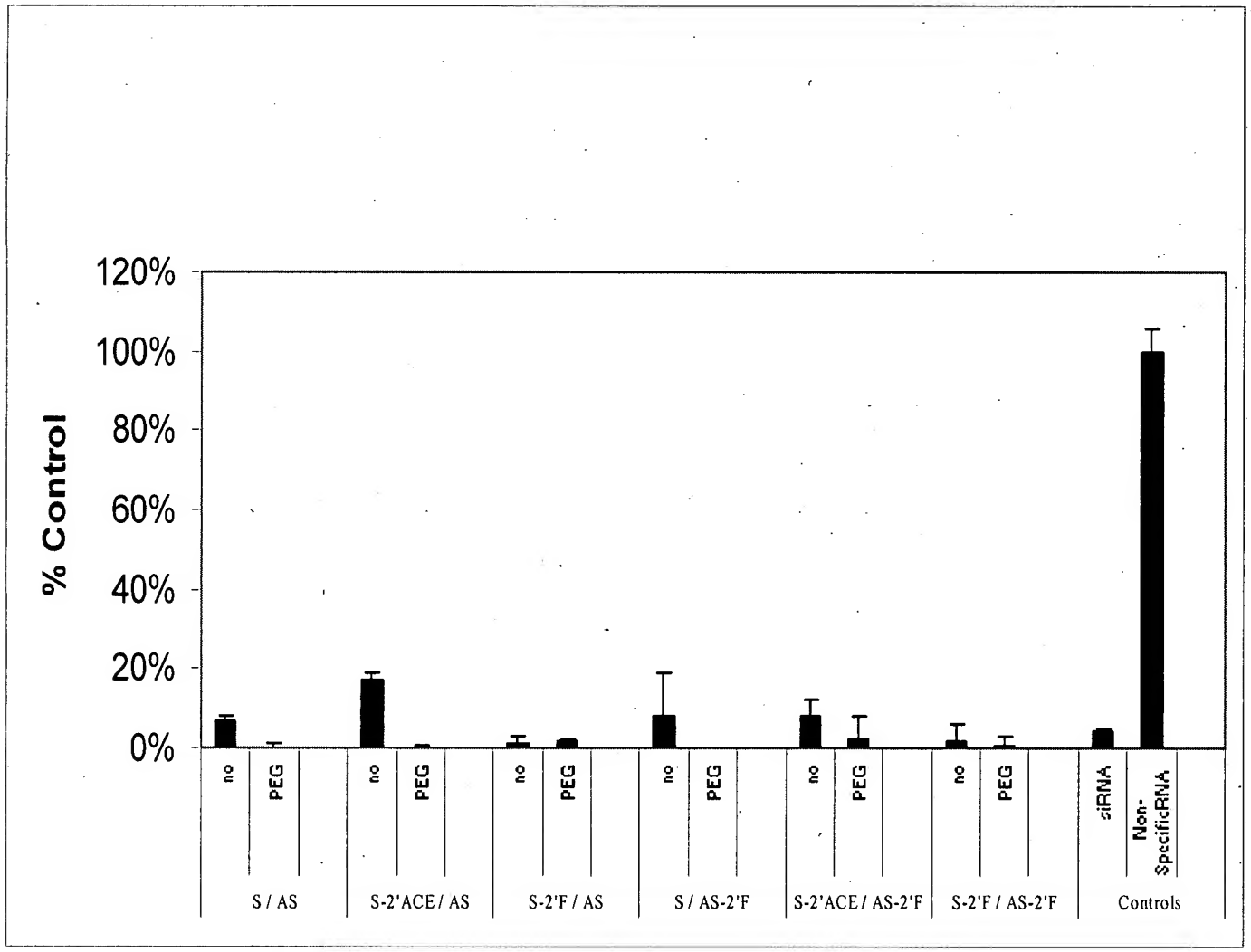



**Figure 10. A PEG conjugate on the 5' end of the sense strand of an siRNA duplex increases siRNA potency**

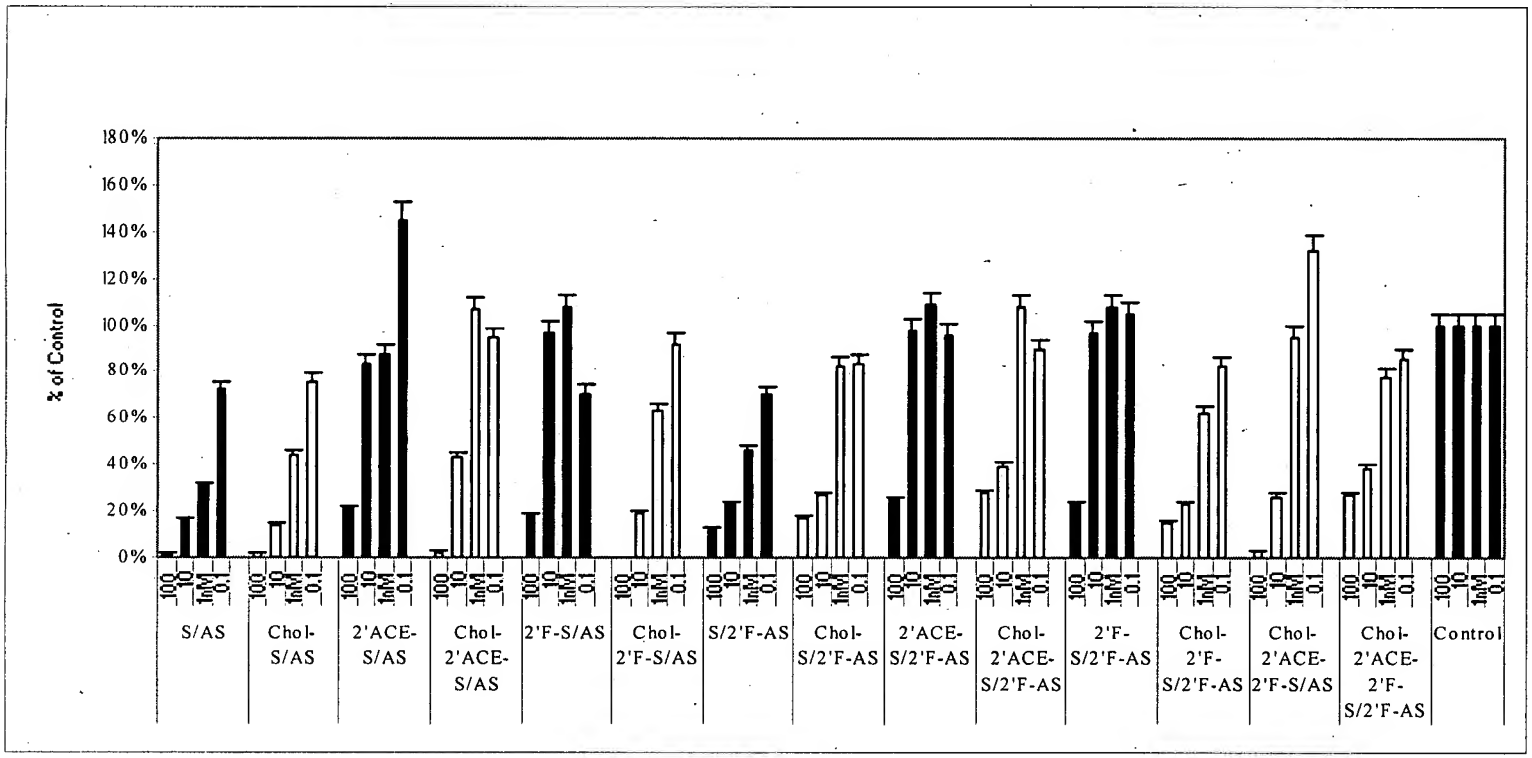



**Figure 11. A sense strand having a 5' cholesterol conjugate results in increased potency and decreased dose of 2'F and orthoester modified oligos**

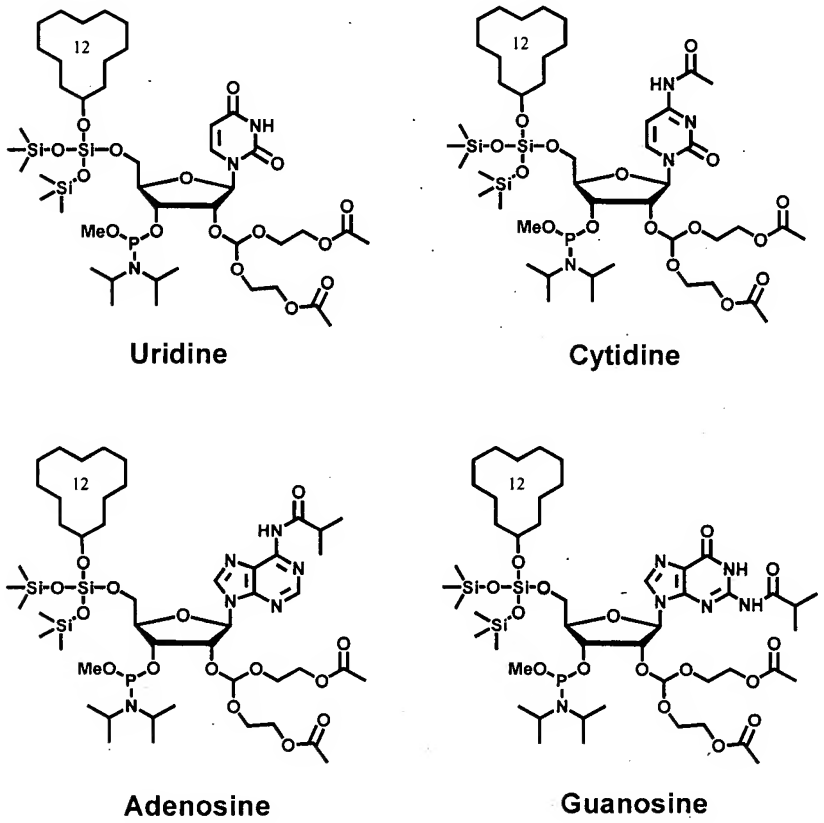
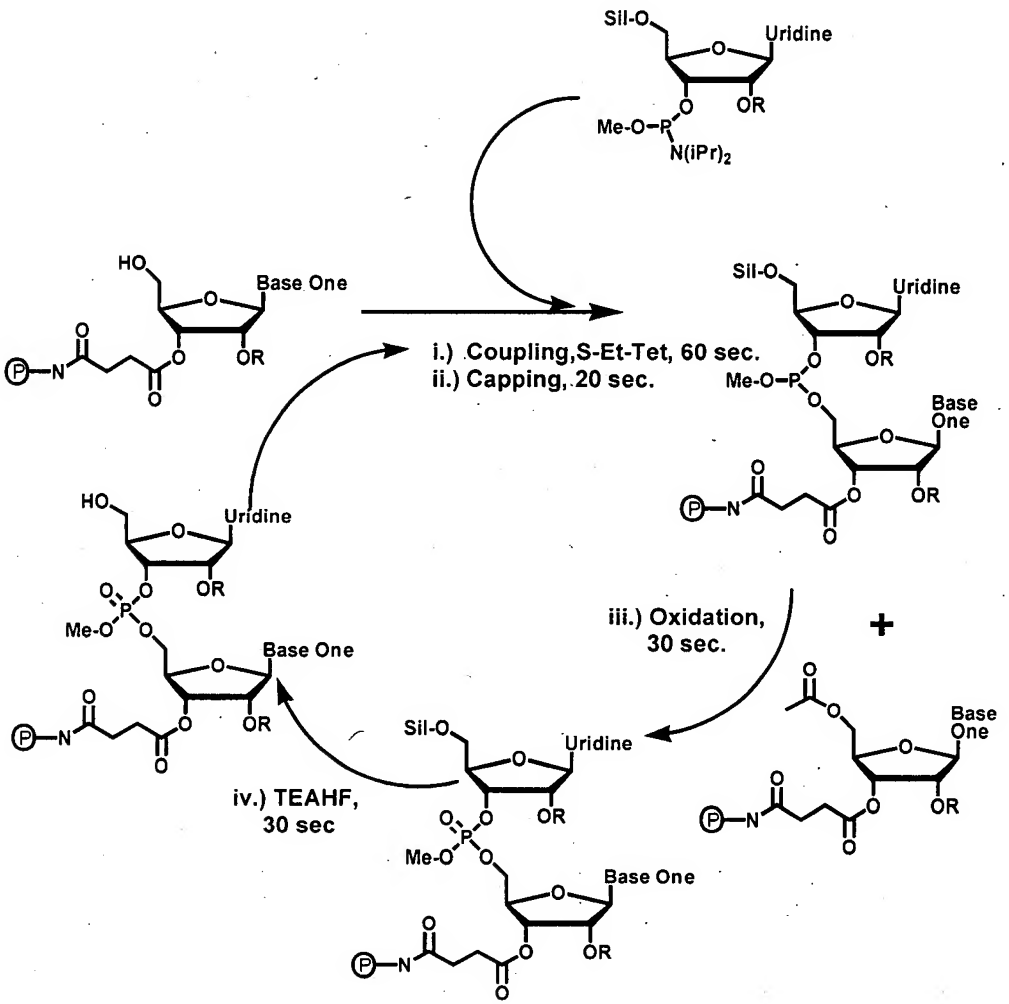



**Figure 12: Protected RNA nucleoside phosphoramidites for Dharmacon 2'-ACE RNA synthesis chemistry.**




**(i)** Couple next nucleoside with S-ethyl-tetrazole catalyst, 60 seconds
**(ii)** Cap unreacted 5′-hydroxyls, 20 seconds
**(iii)** Oxidize phosphorus linkage (t-butyl hydroperoxide)
**(iv)** 5′-deprotection with triethylammonium fluoride ions (TEAHF), 30 seconds

**Figure 13: Outline of Dharmacon RNA Synthesis Cycle.**

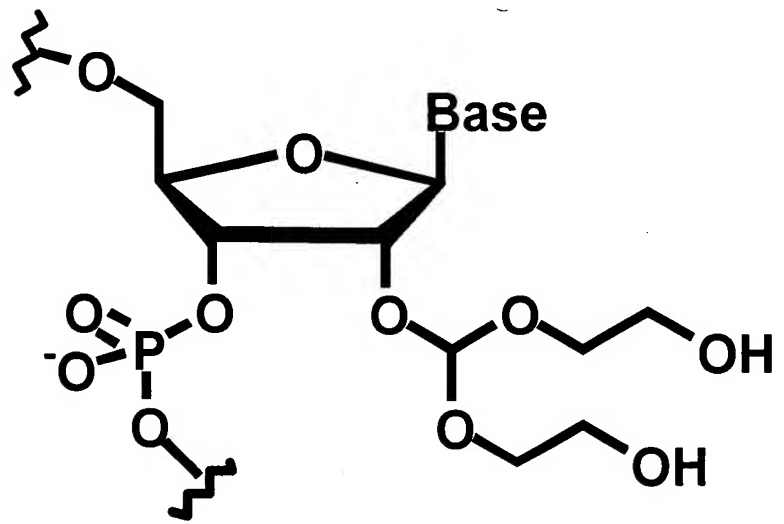



**Figure 14: Structure of 2'-ACE protected RNA immediately prior to 2'-deprotection.**

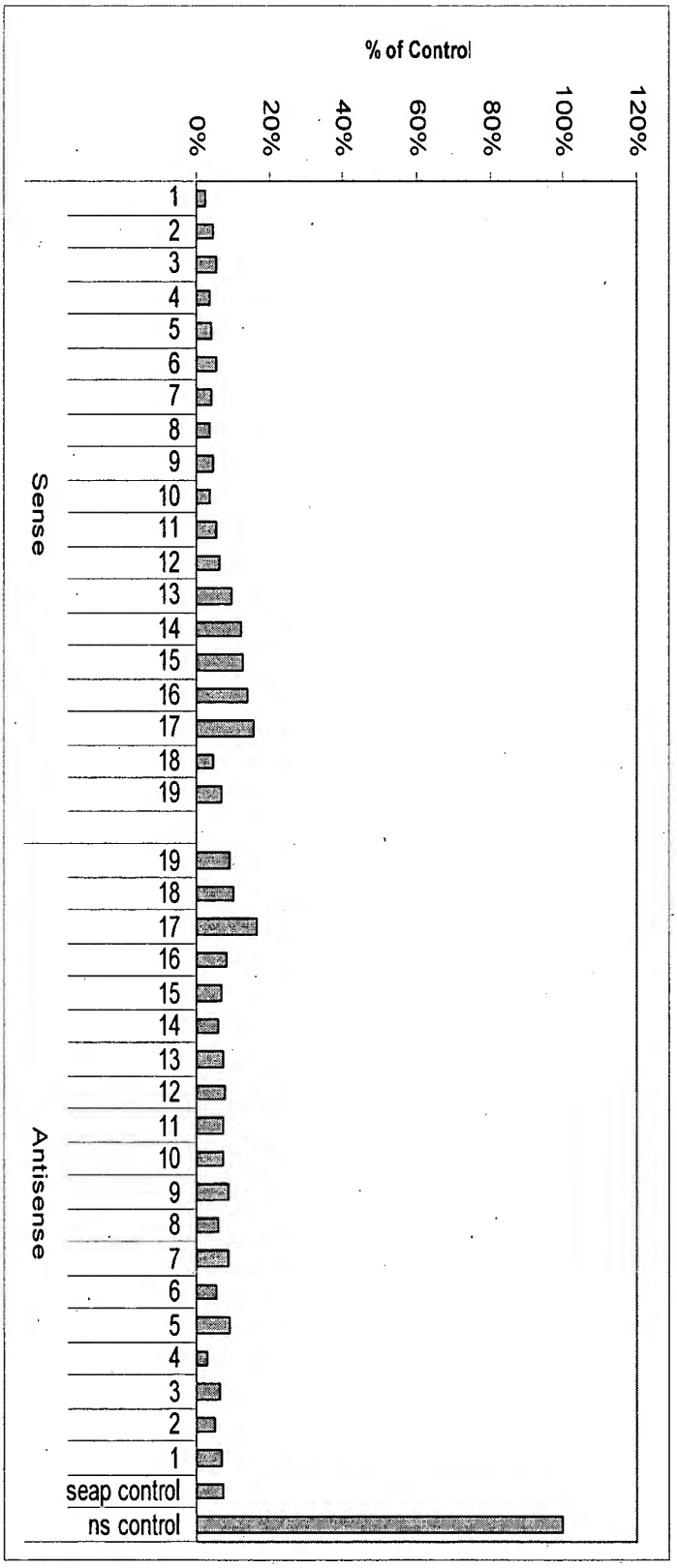



**Figure 15A: Single Deoxynucleotide Modification**

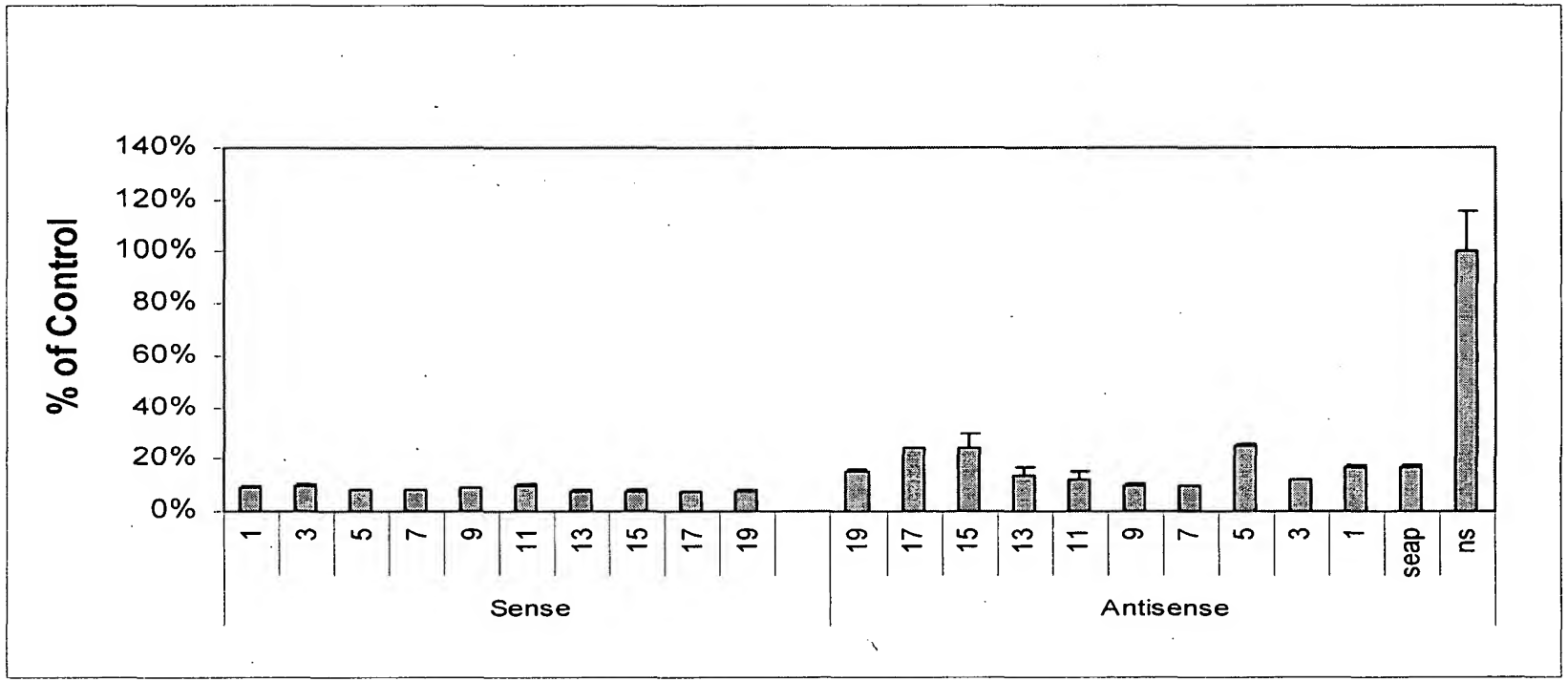



**Figure 15B: Two Deoxynucleotide Modifications in Tandem**

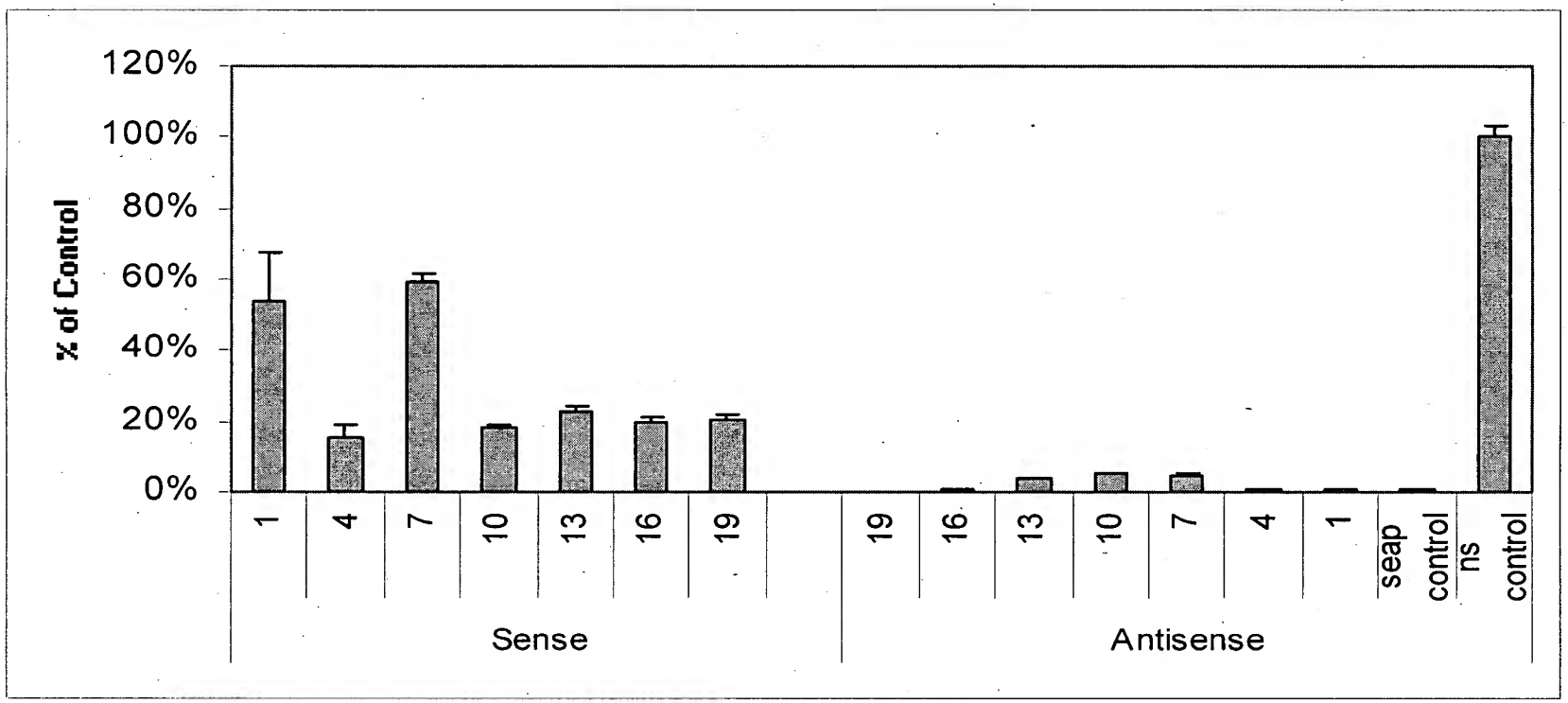



**Figure 15C: Three Deoxynucleotide Modifications in Tandem**

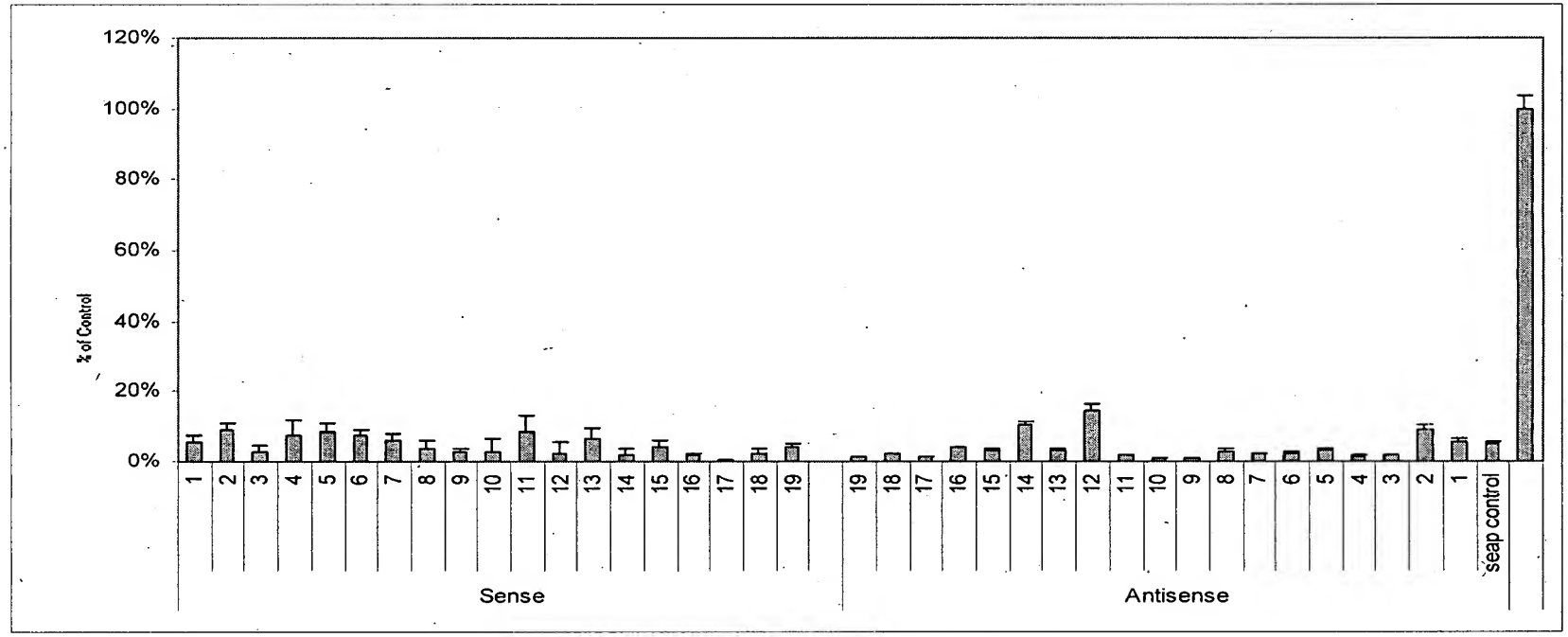



**Figure 16A: Single 2'-O-Methyl Modification**

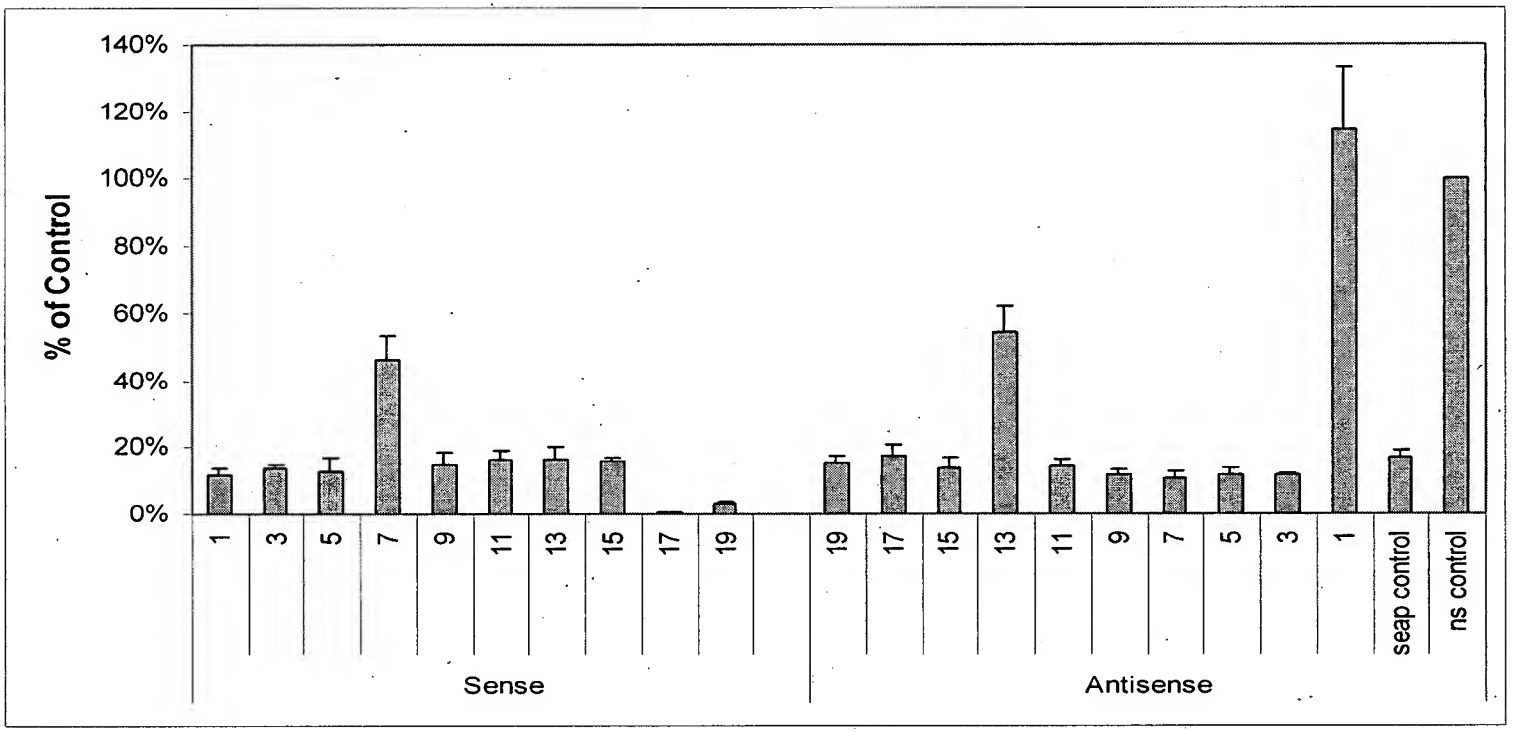



**Figure 16B: Two 2'-O-Methyl Modifications in Tandem**

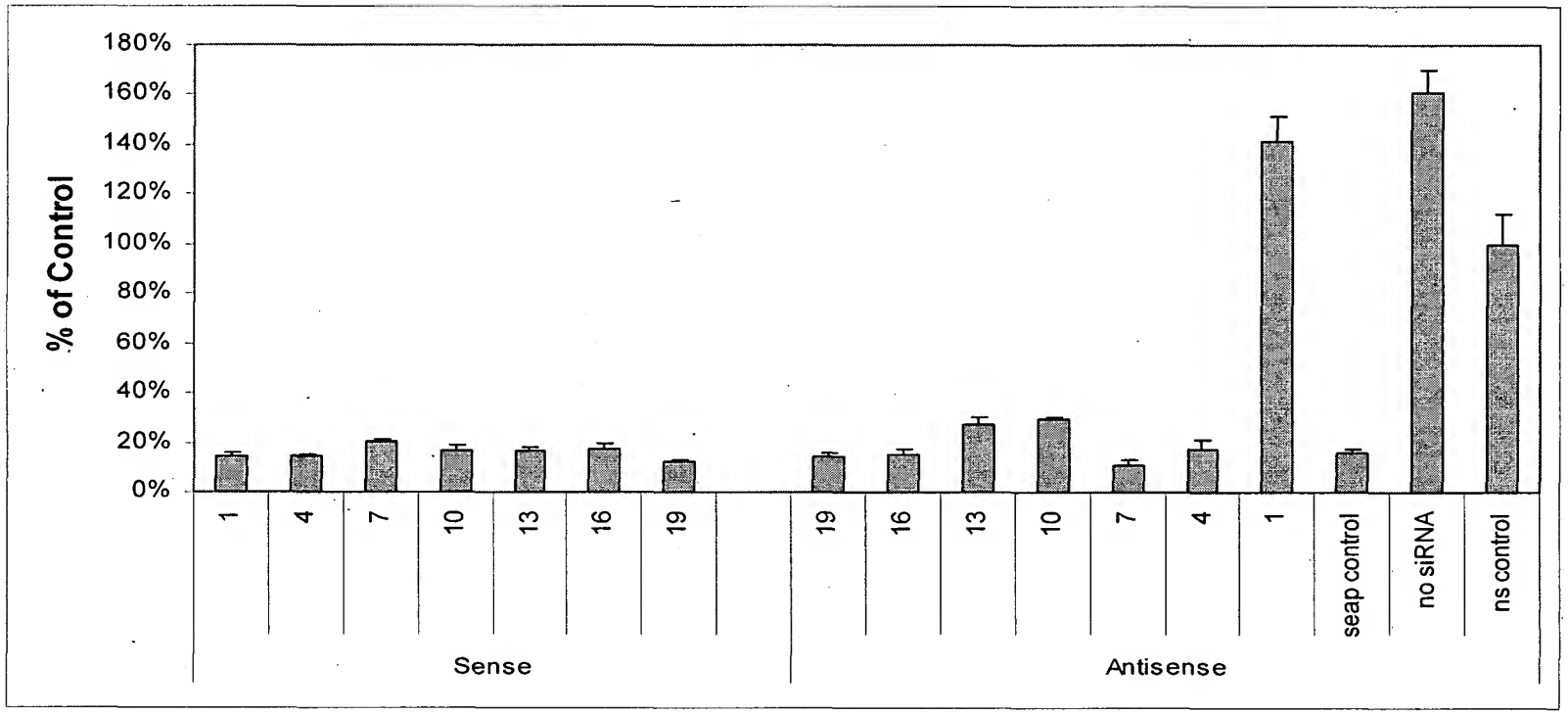



**Figure 16C: Three 2'-O-Methyl Modifications in Tandem**

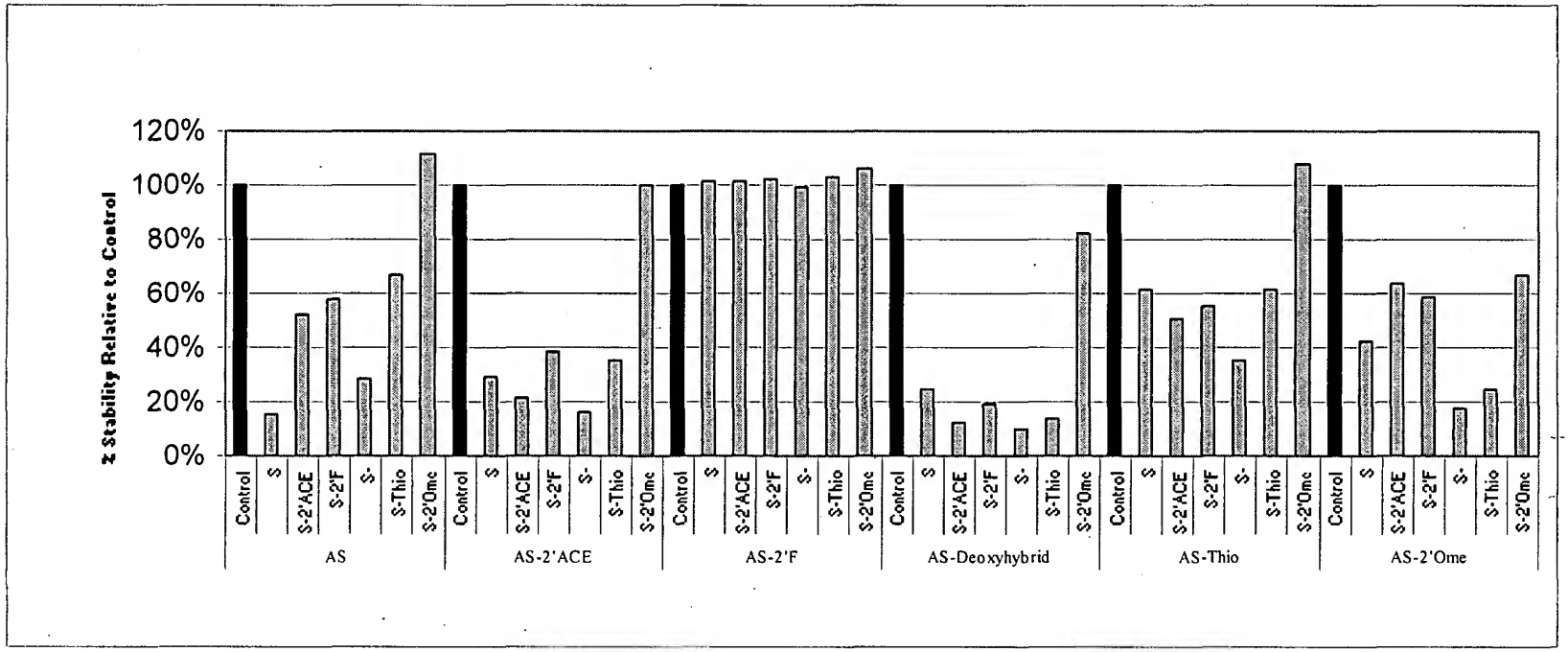



**Figure 17: Stability Screen for One Hour Incubation in Media**

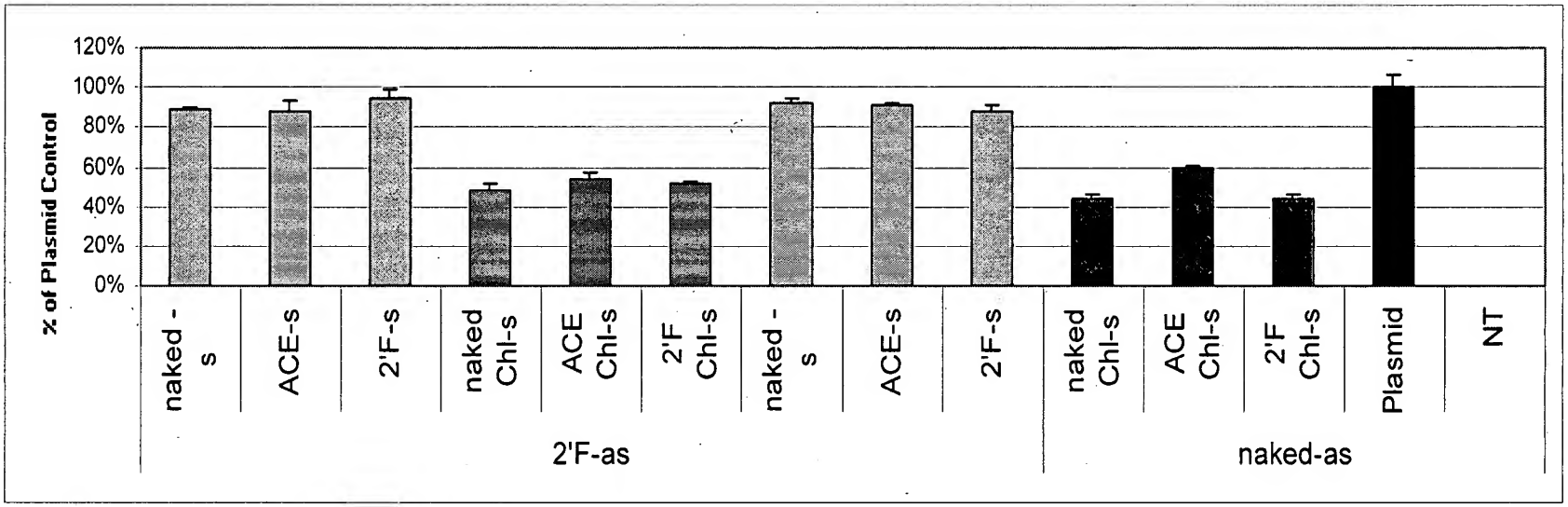



**Figure 18: Effect of Cholesterol Modification on Passive Delivery of siRNA**

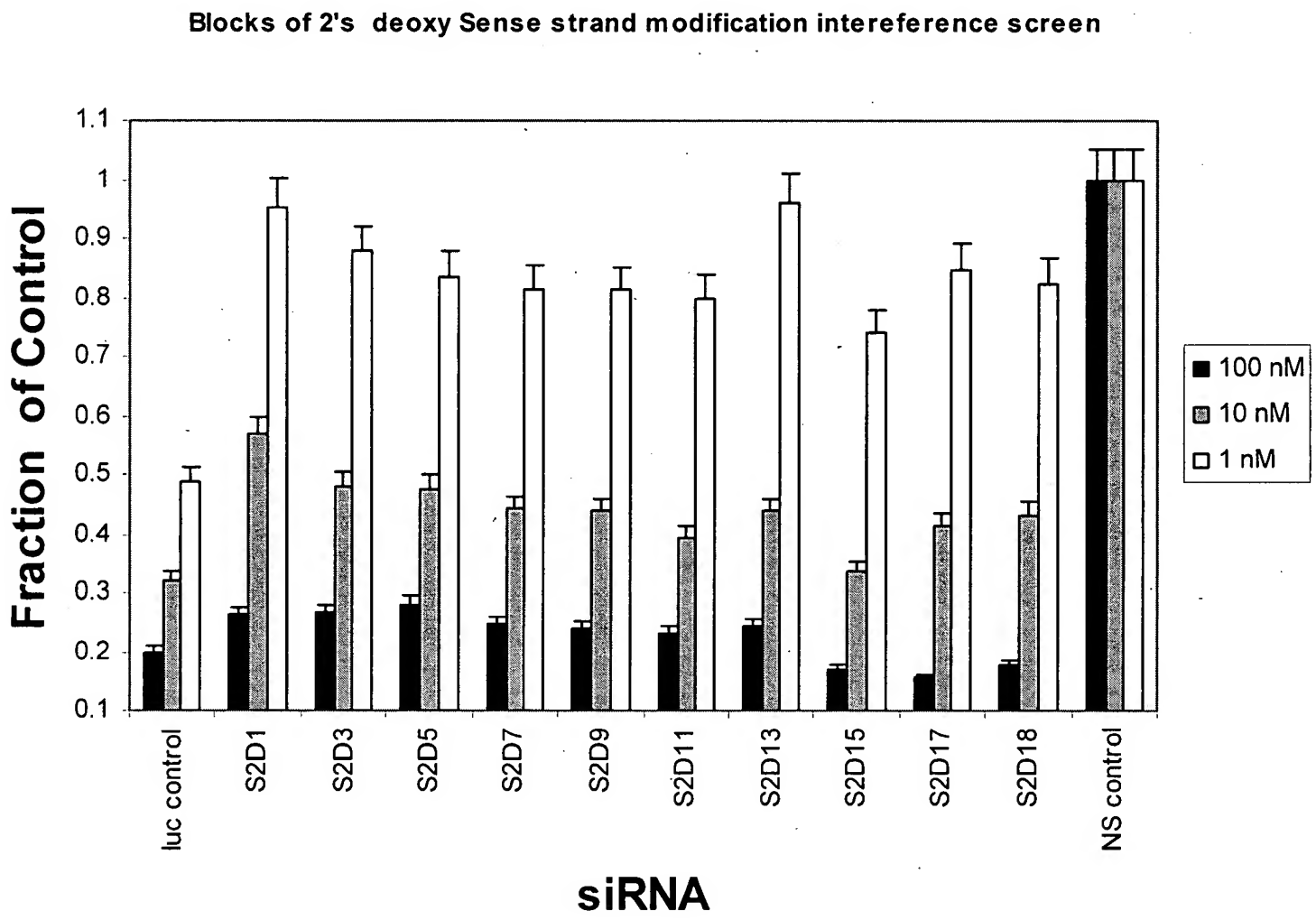



**Figure 19: Modification intereference screen: blocks of 2 deoxy in the sense strand**

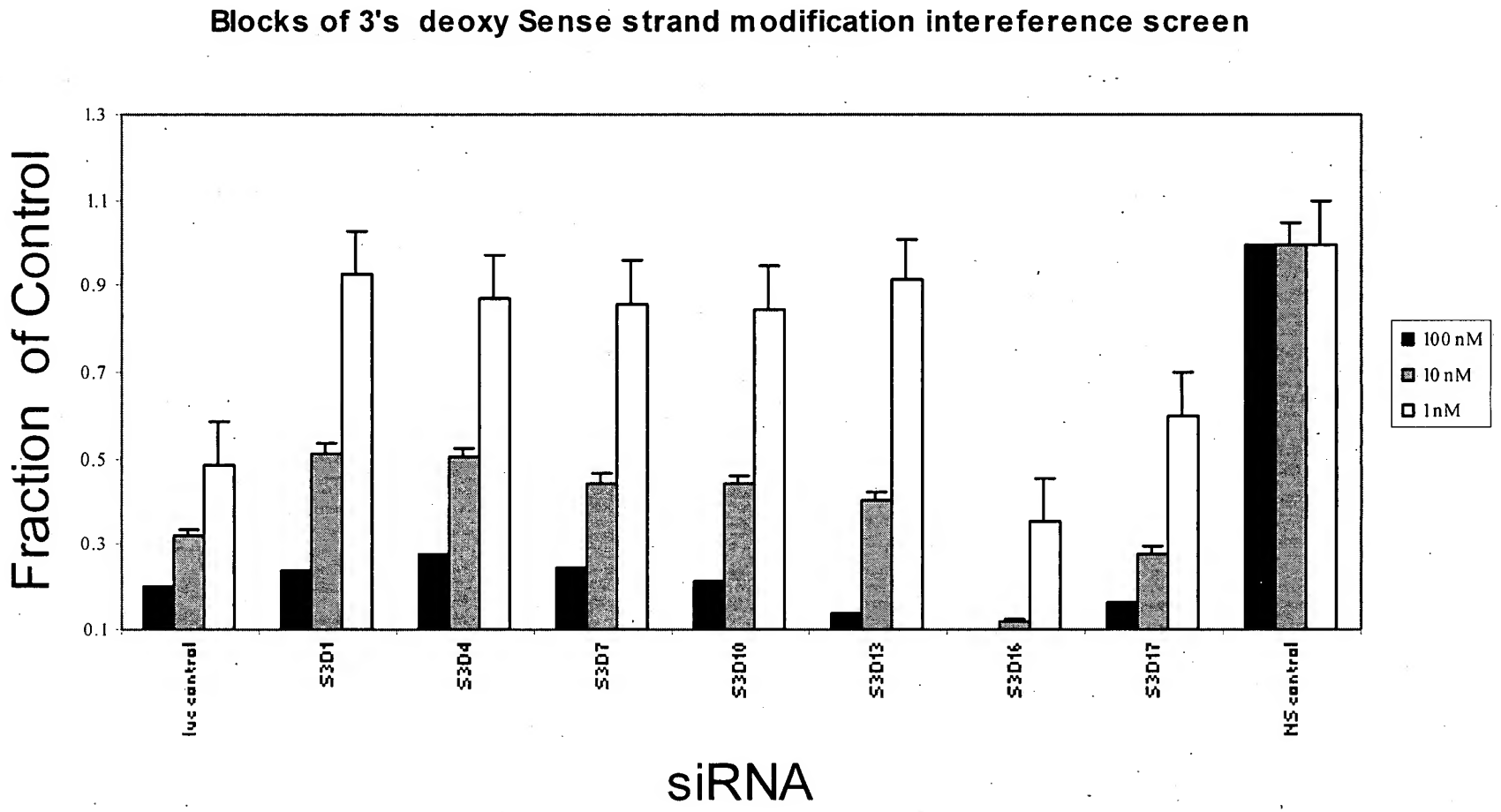



**Figure 20: Modification intereference screen: blocks of 3 deoxy in the sense strand**

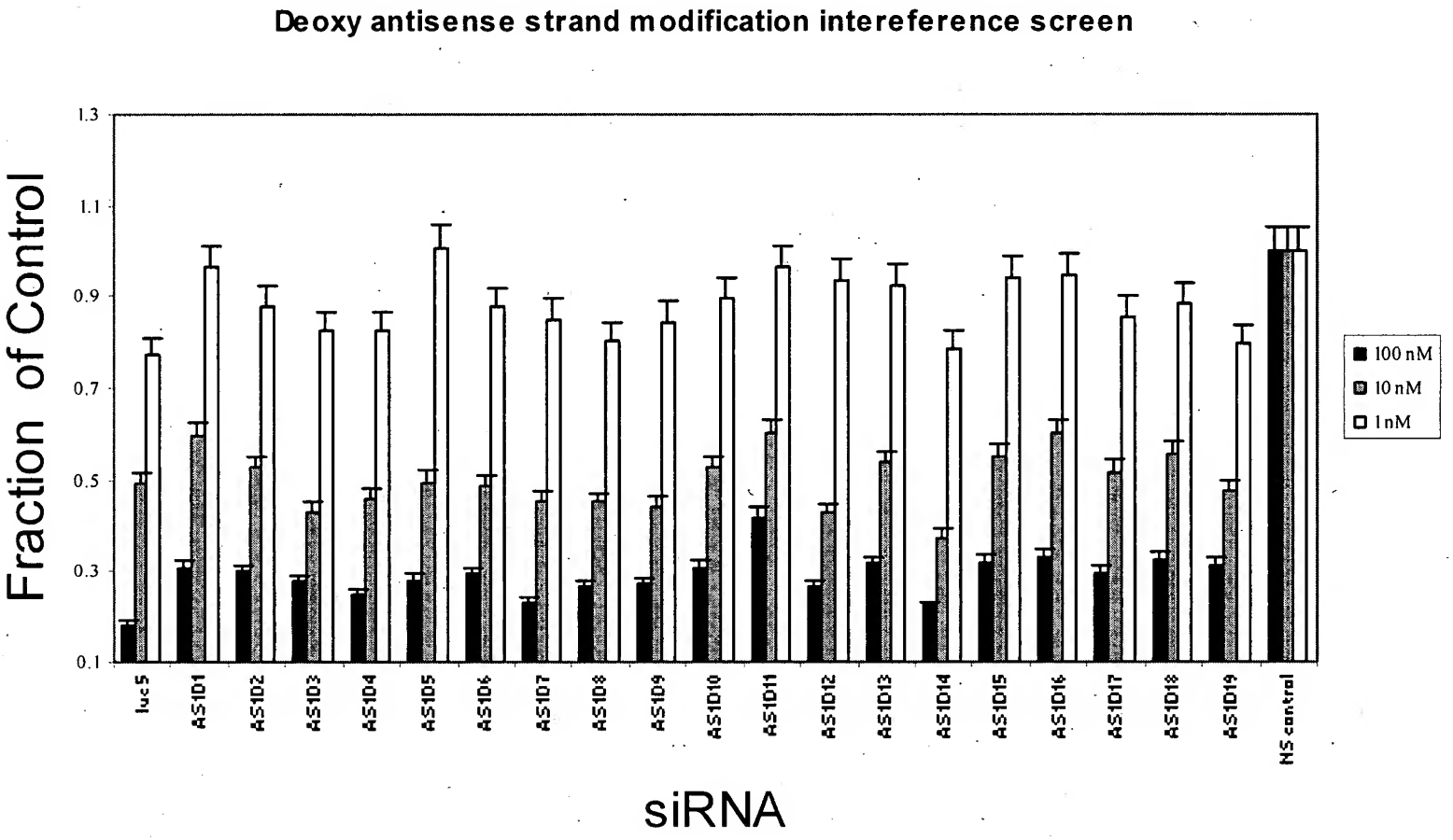



**Figure 21: Modification intereference screen: deoxy in the antisense strand**

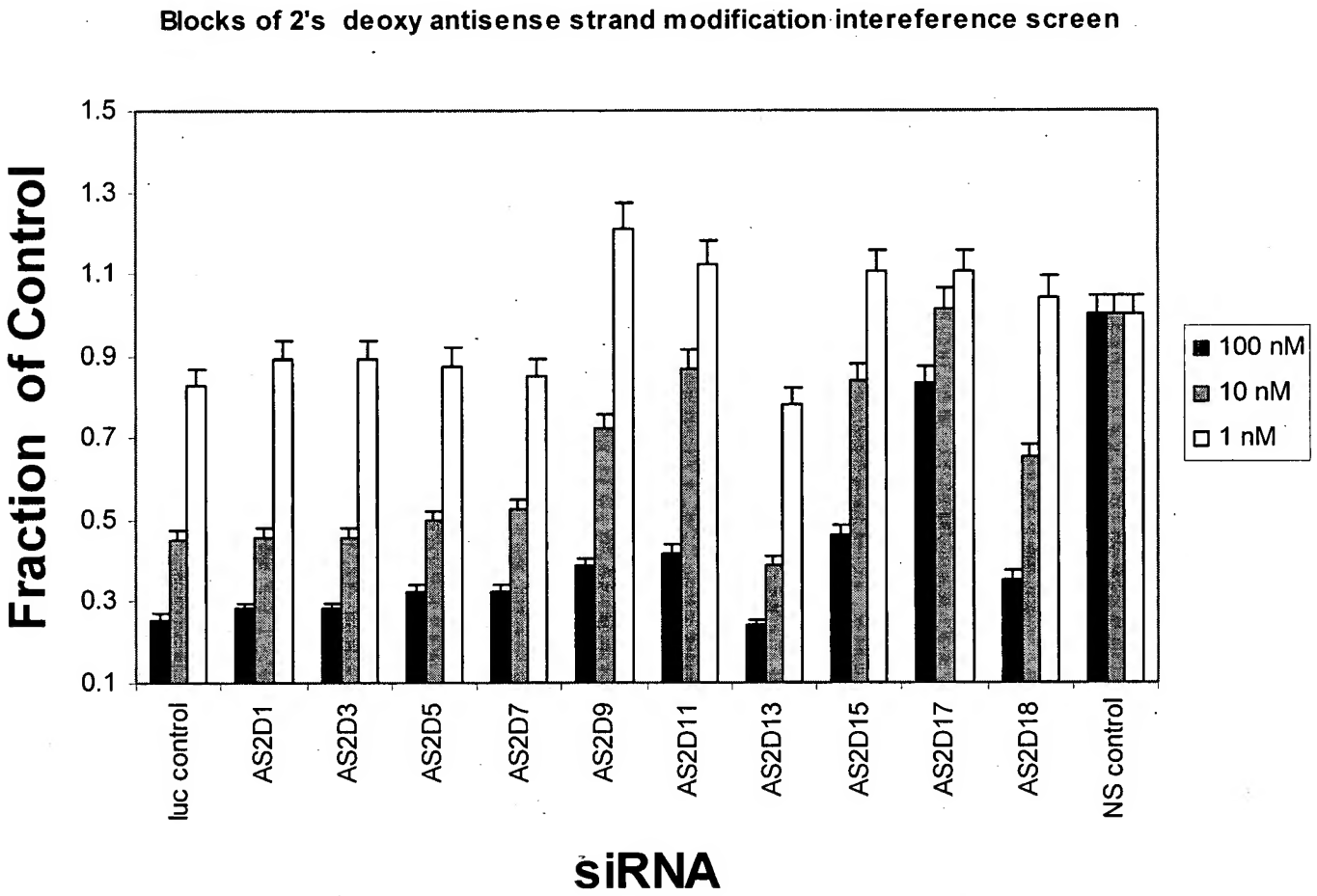



**Figure 22: Modification intereference screen: blocks of 2 deoxy in the antisense strand**

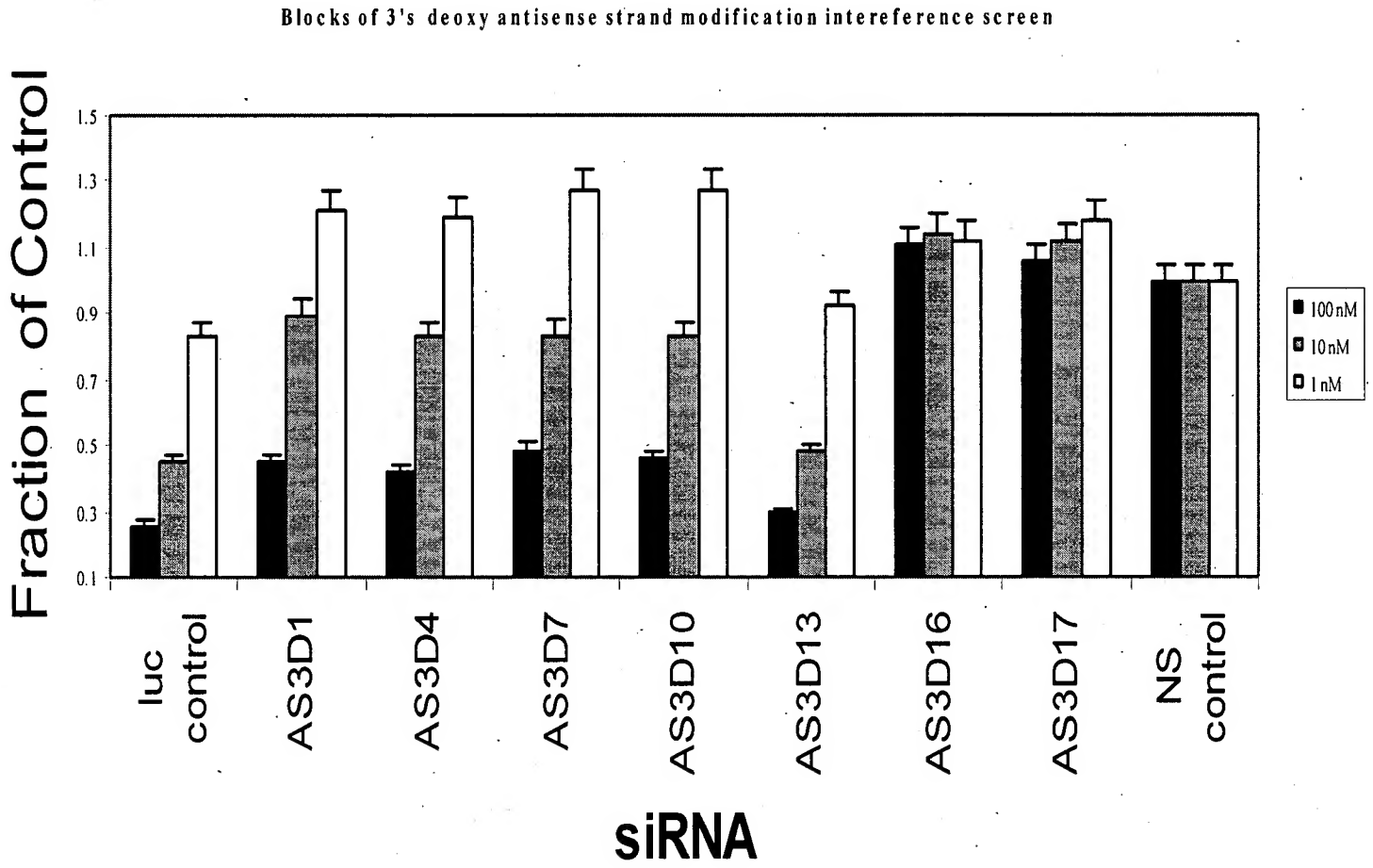



**Figure 23: Modification intereference screen: blocks of 3 deoxy in the antisense strand**

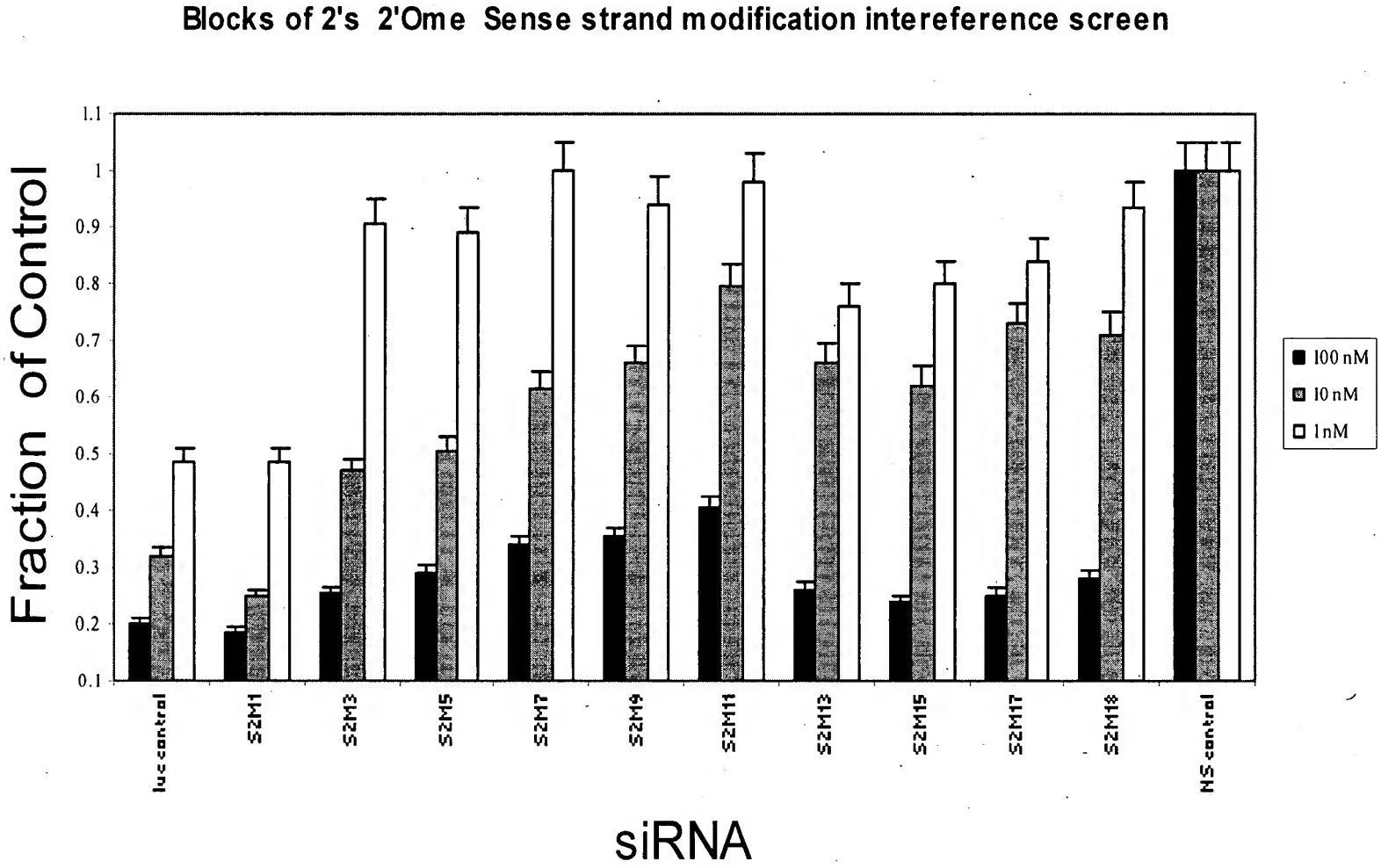



**Figure 24: Modification intereference screen: blocks of 2 methoxy in the sense strand**

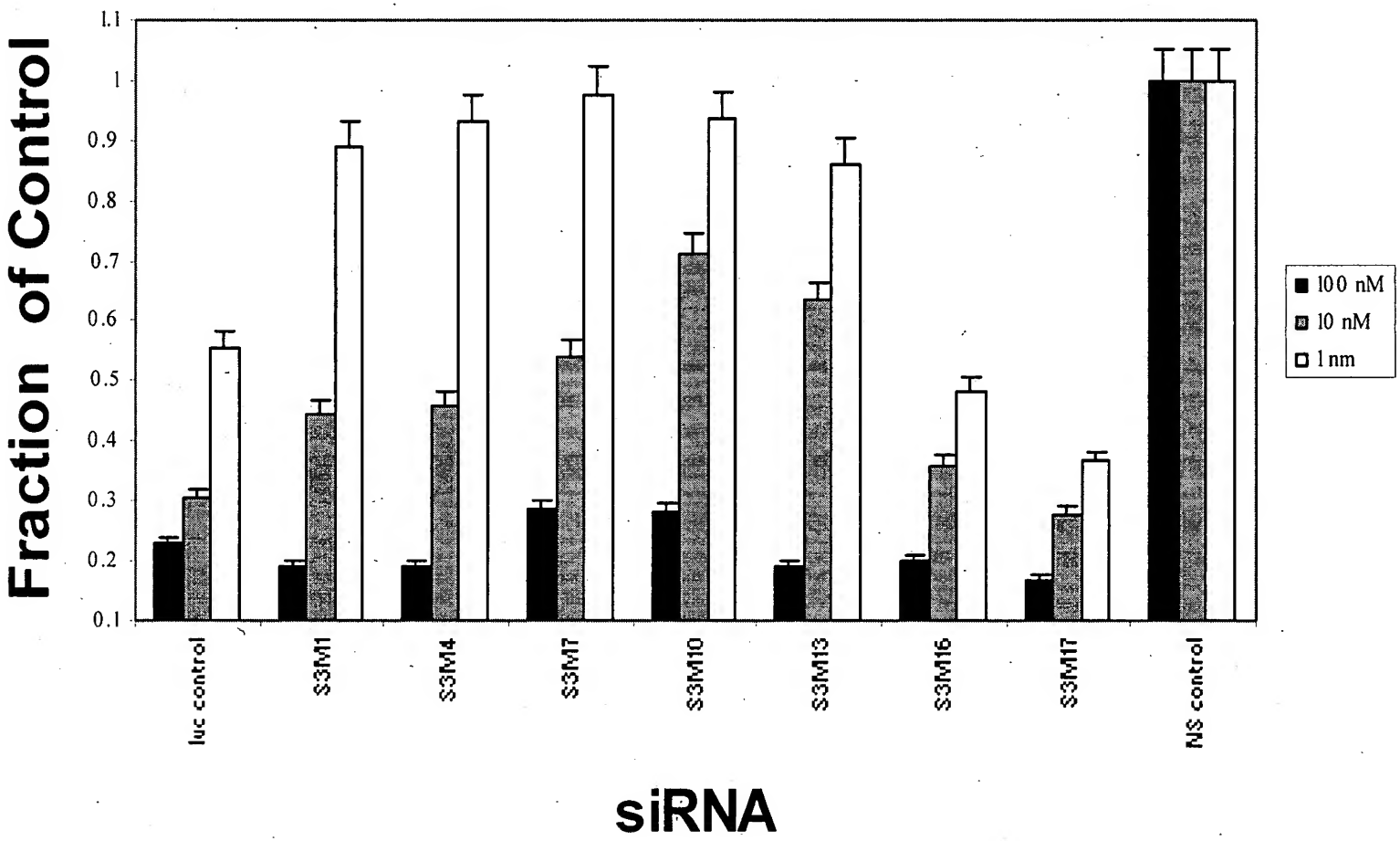



**Figure 25: Modification intereference screen: blocks of 3 methoxy in the sense strand**

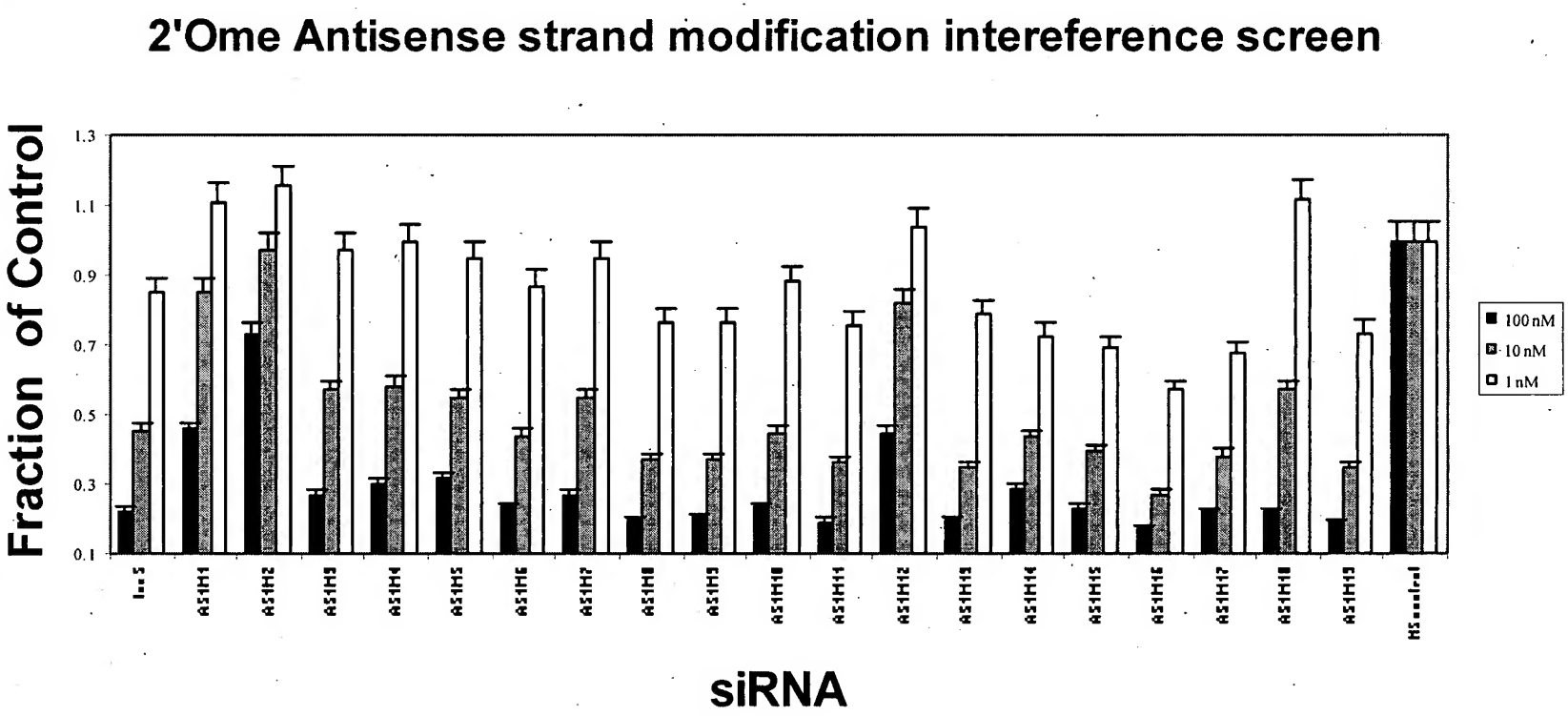



**Figure 26: Modification intereference screen: methoxy in the antisense strand**

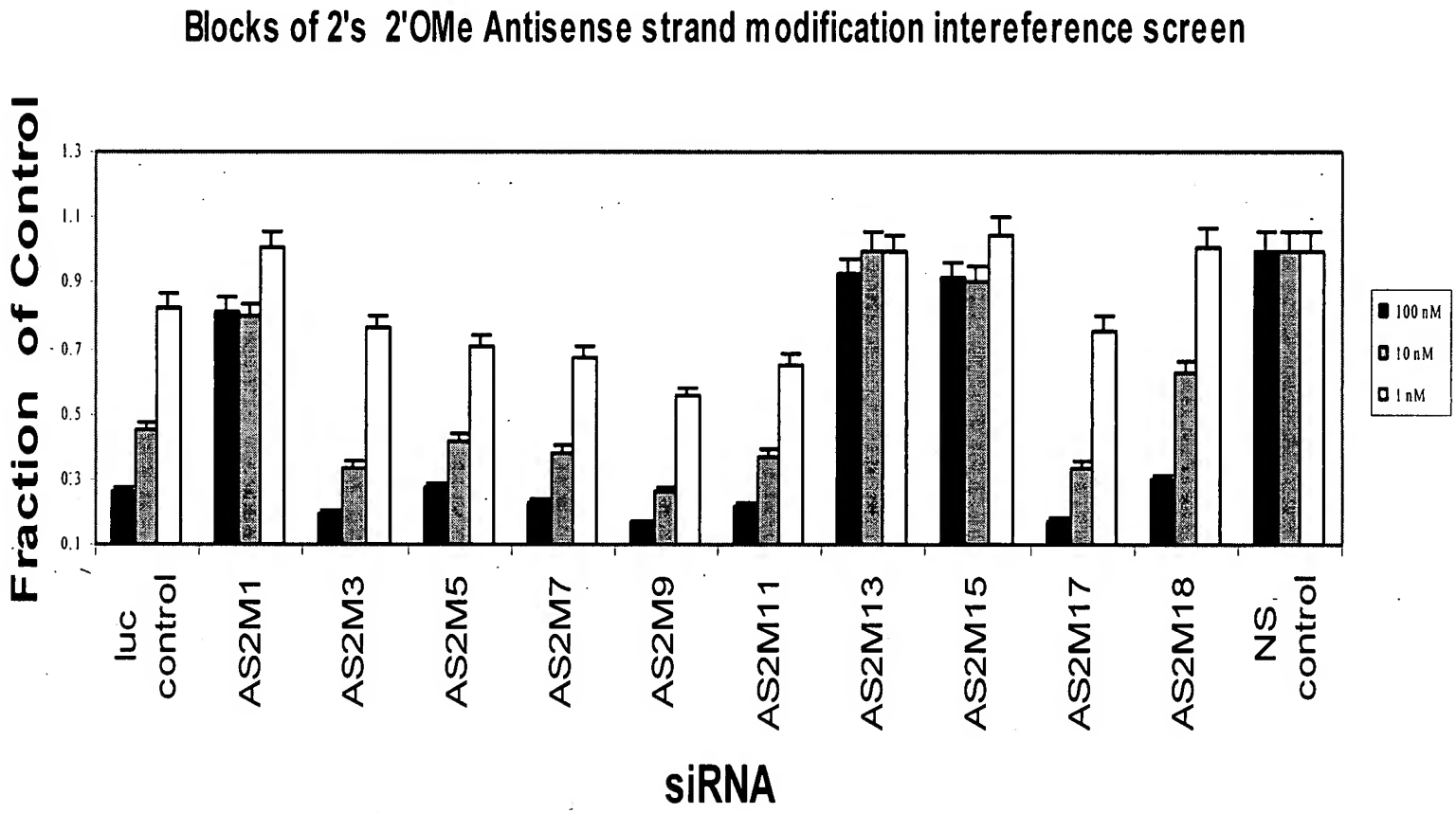



**Figure 27: Modification intereference screen: blocks of 2 methoxy in the antisense strand**

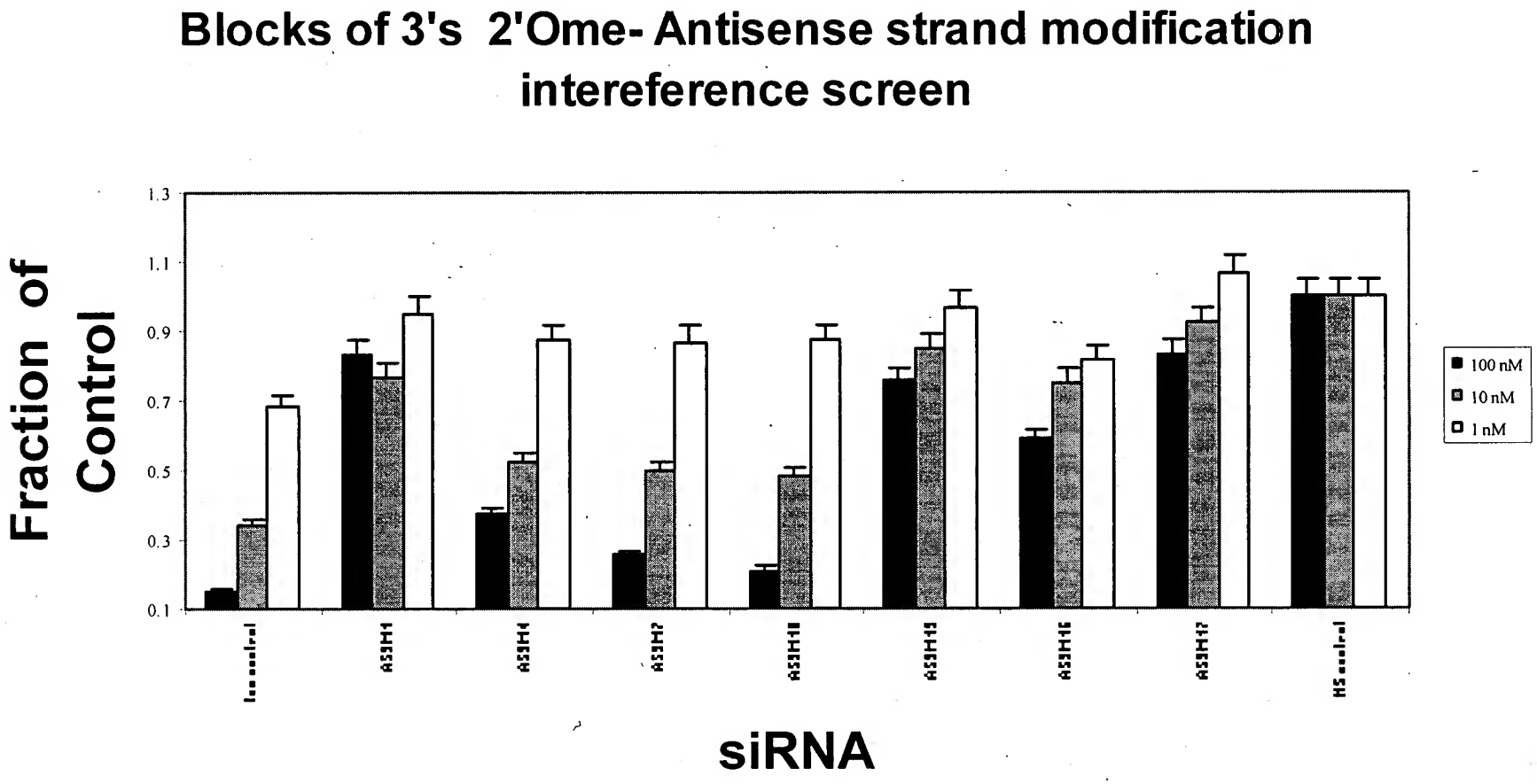



**Figure 28: Modification intereference screen: blocks of 3 methoxy in the antisense strand**

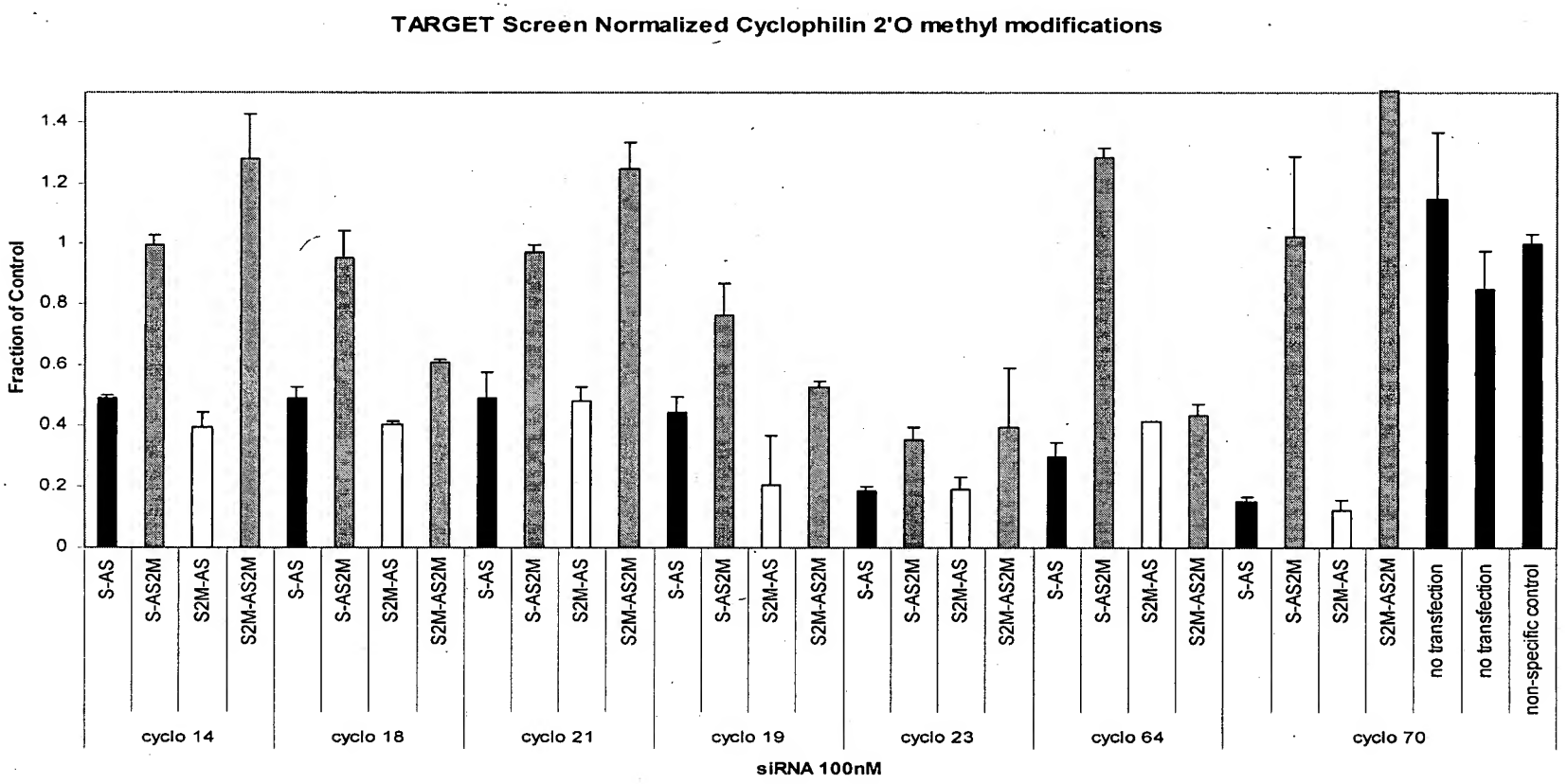



**Figure 29: Presence of the 2- 2'Ome modifications result on the 5'AS strand interfere with functionality in human Cyclophilin**

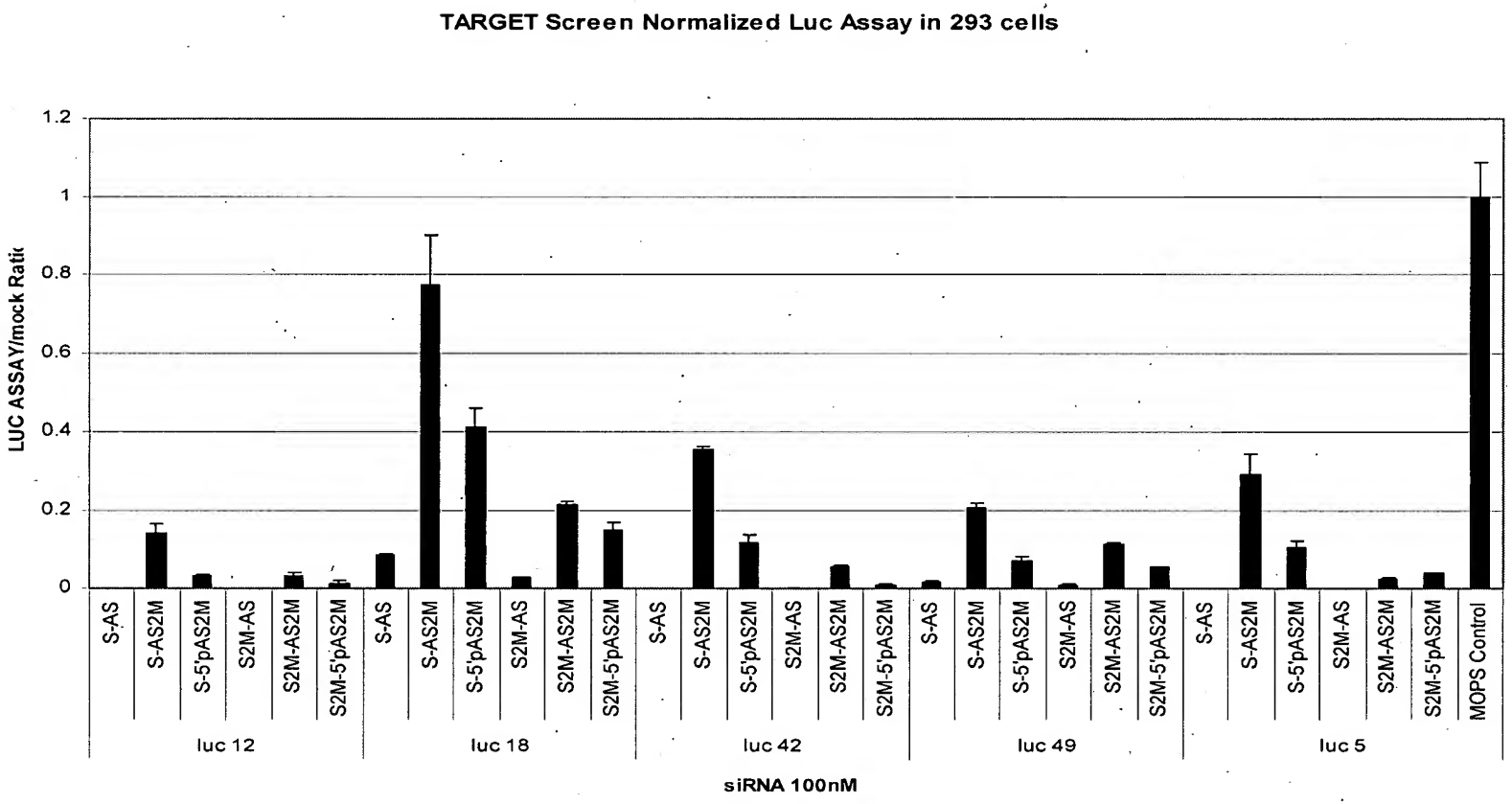



**Figure 30: Presence of the 2- 2'Ome modifications result on the 5'AS strand interfere with functionality in the Firefly Luciferase**

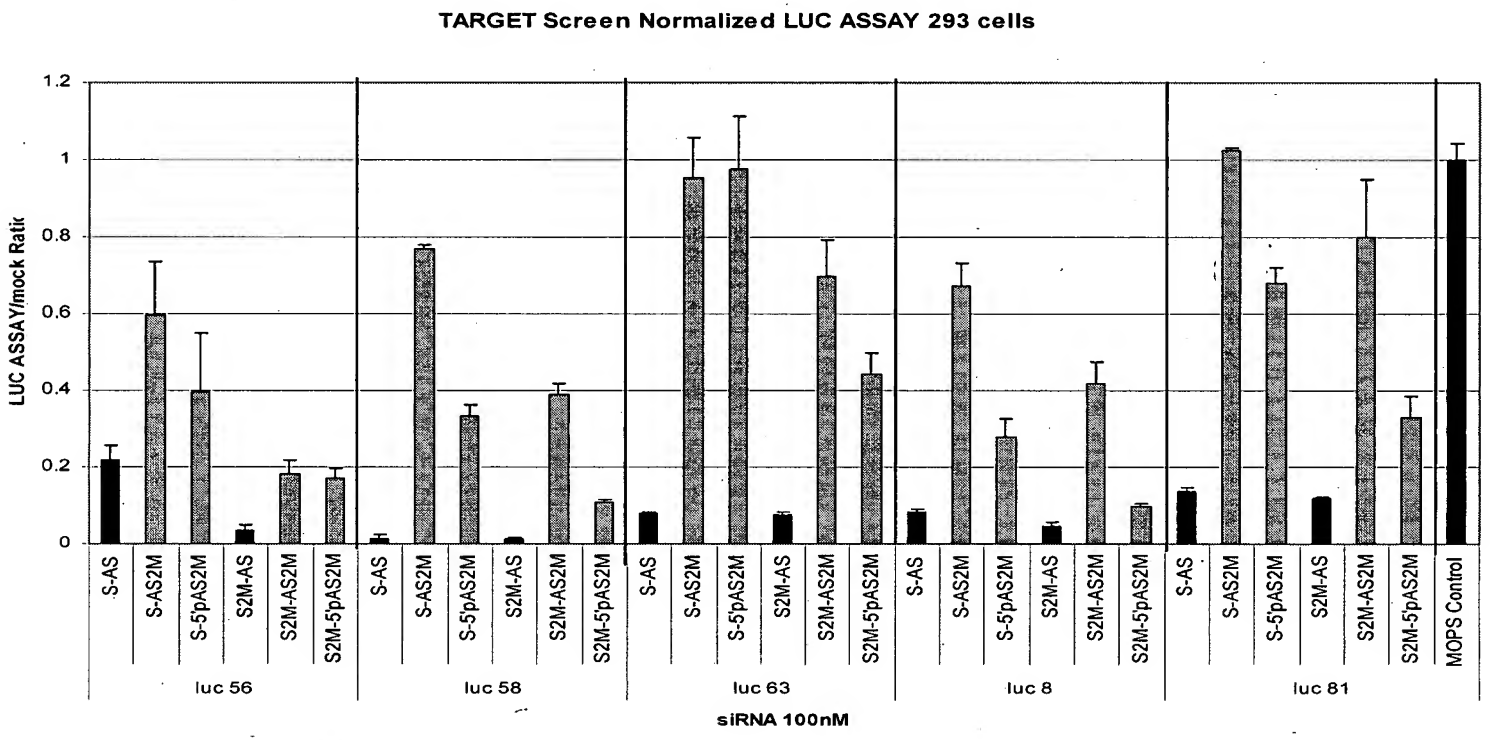



**Figure 31: Presence of the 2- 2'Ome modifications result on the 5'AS strand interfere with functionality**

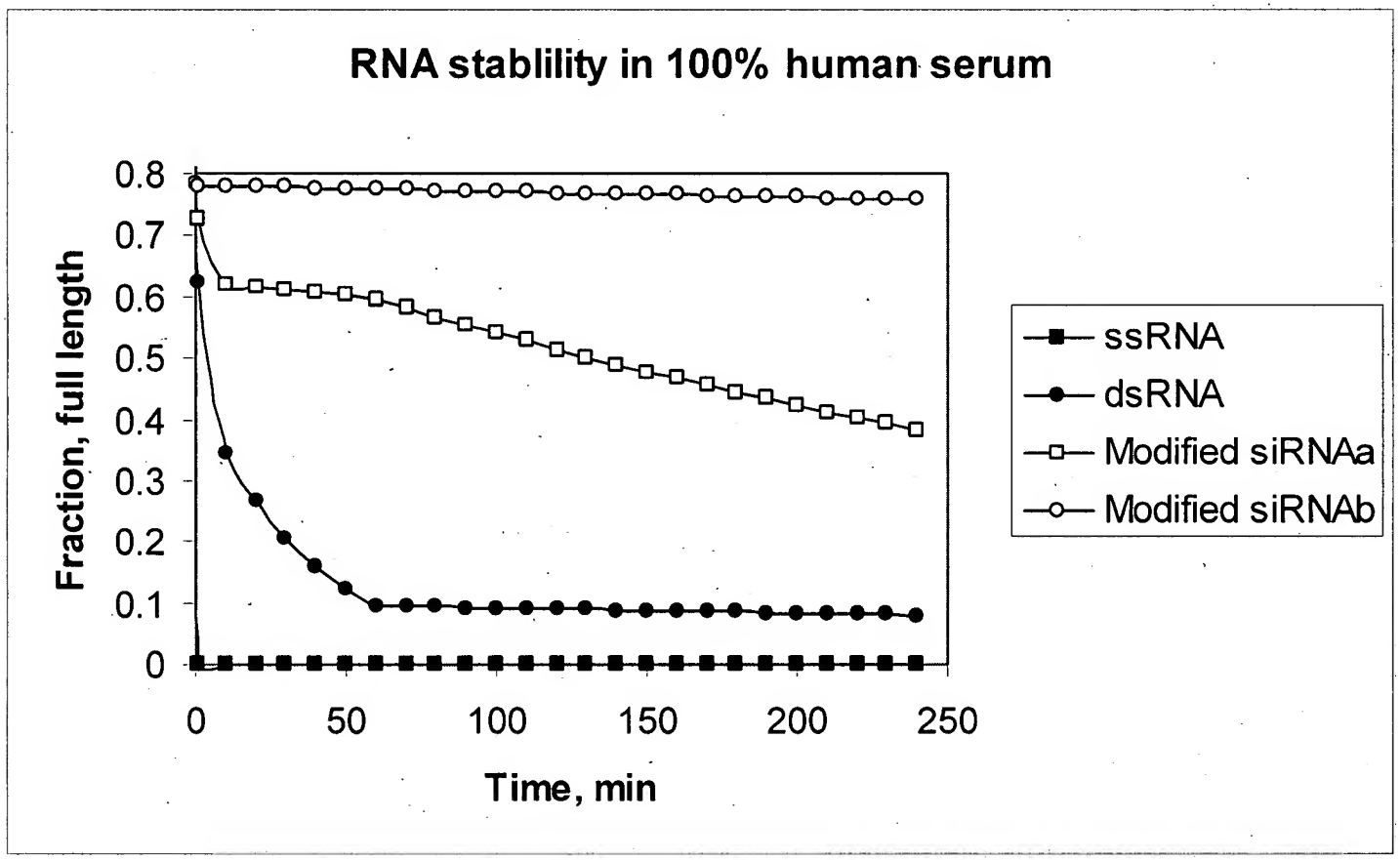



**Figure 32: siRNA stability in 100% human serum**

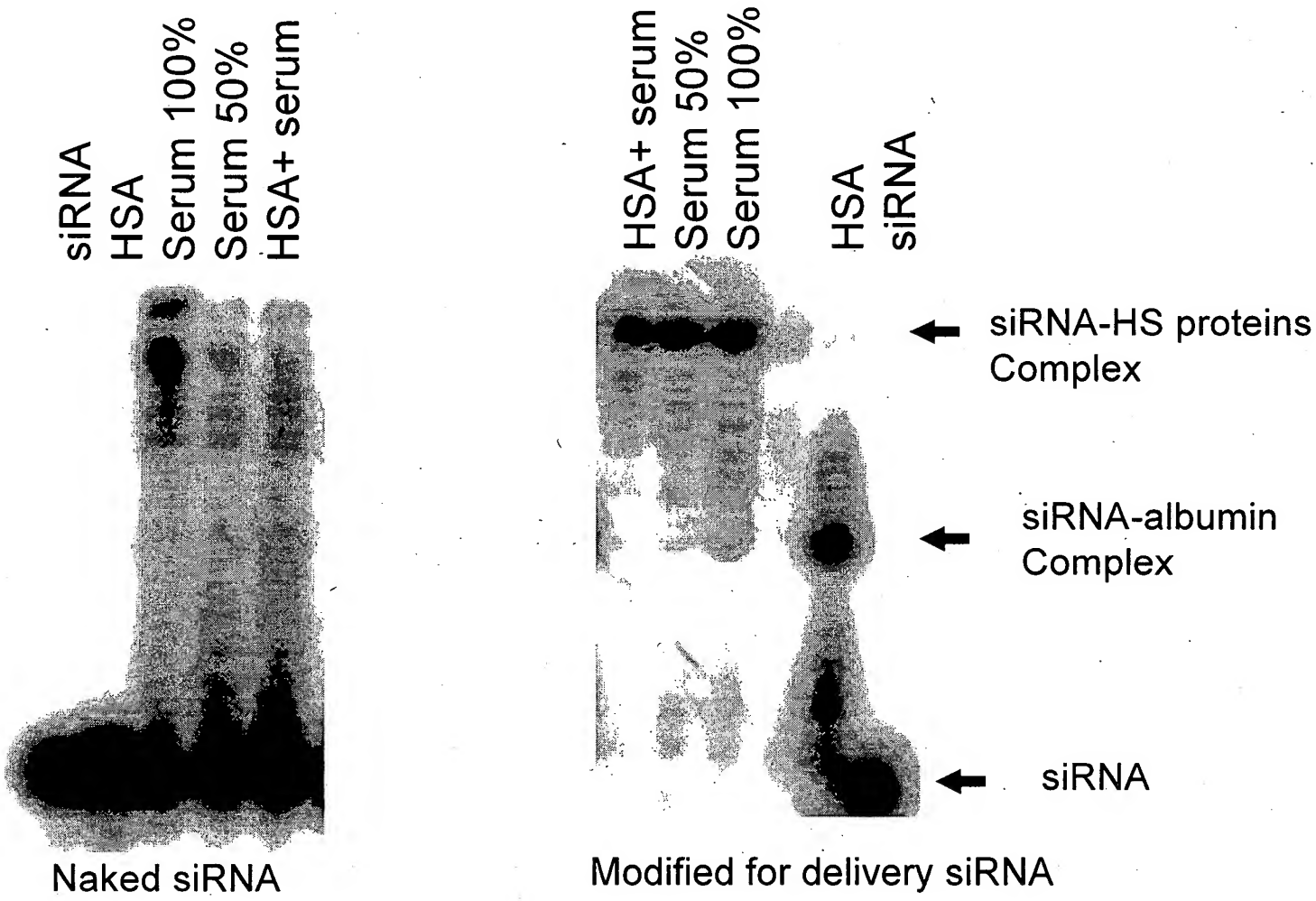



**Figure 33: siRNA- cholesterol conjugates has increased affinity to albumin and other serum proteins**

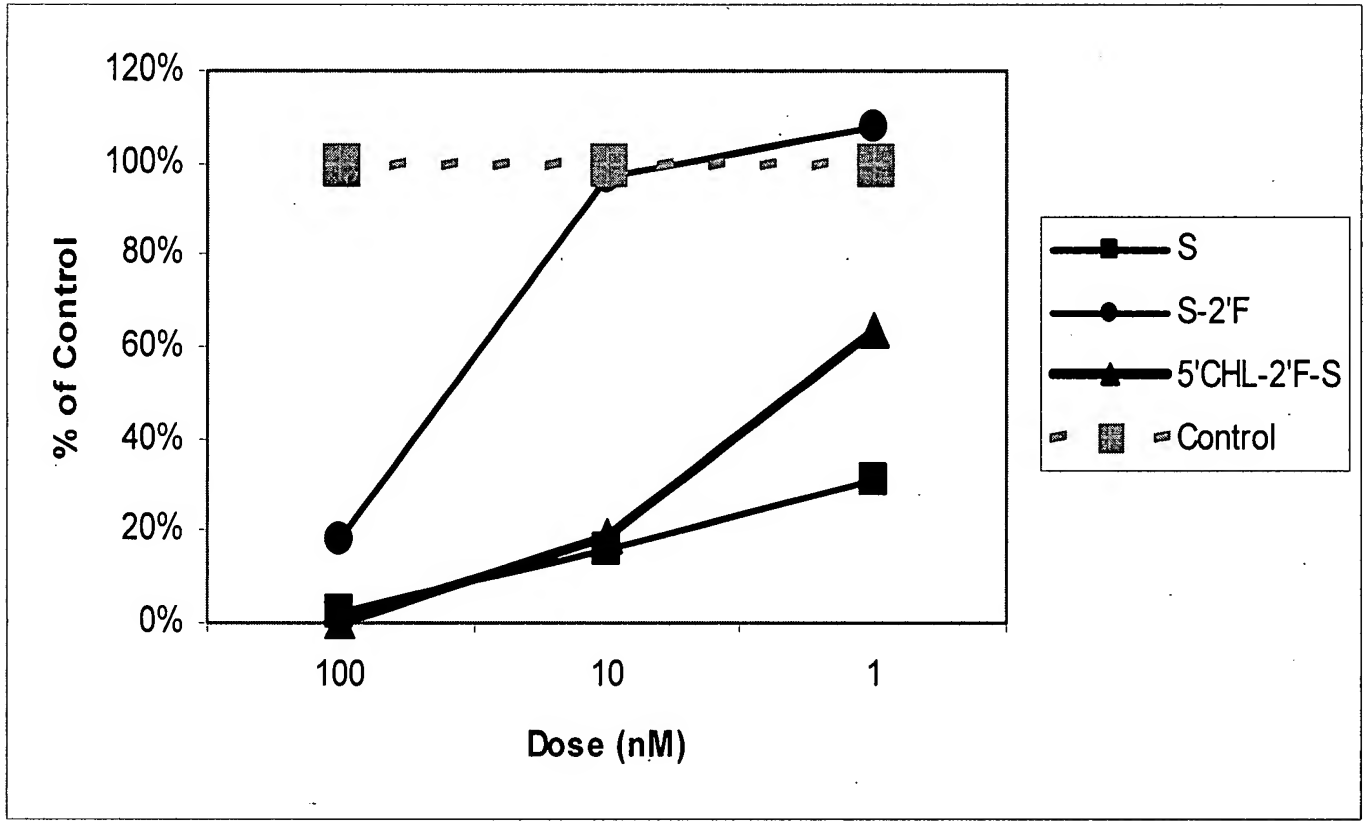



**Figure 34: Small Molecule Conjugates Maintain and Accentuate the Potency of Modified siRNA**

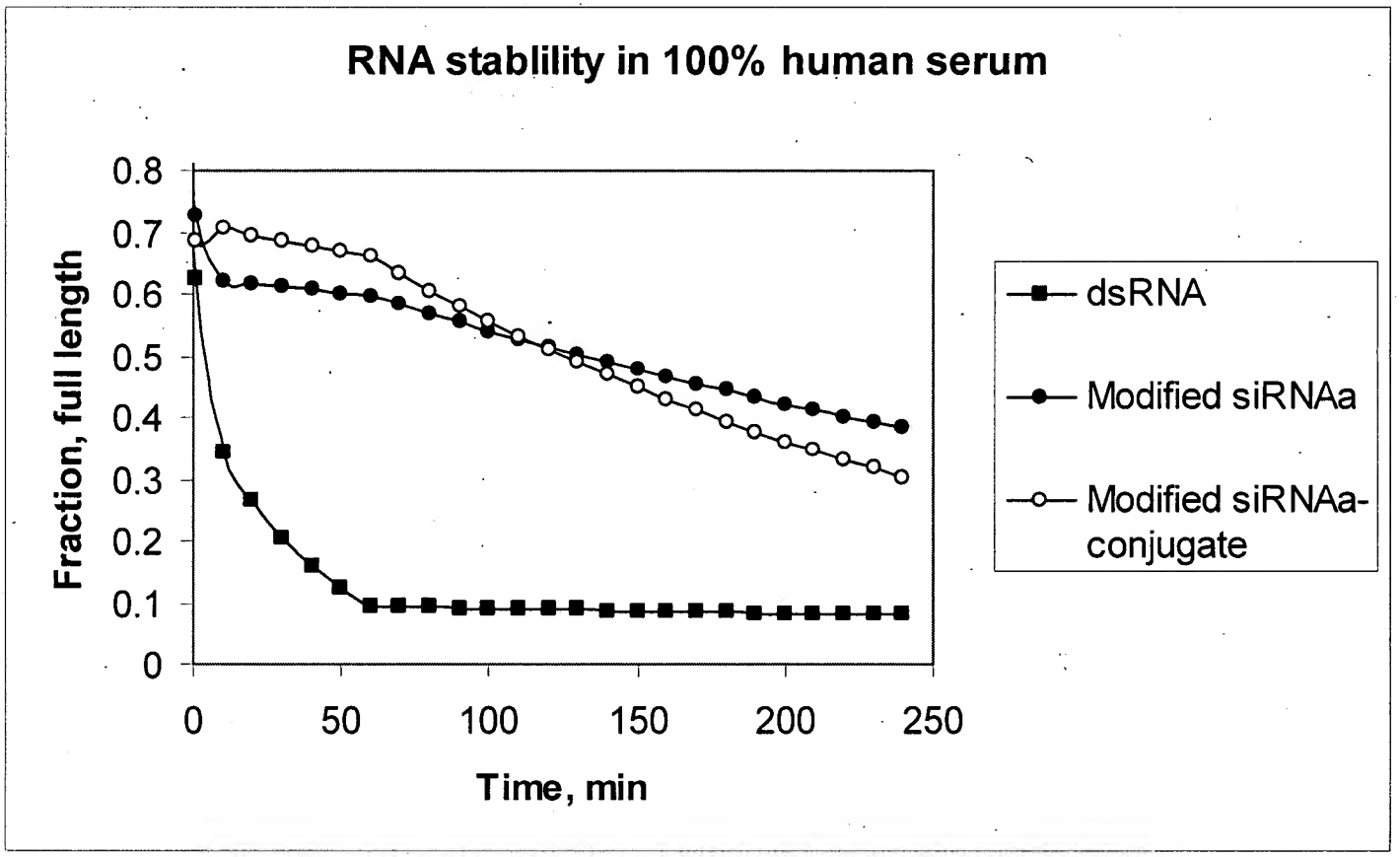



**Figure 35: Stability of siRNA conjugates in Human serum**

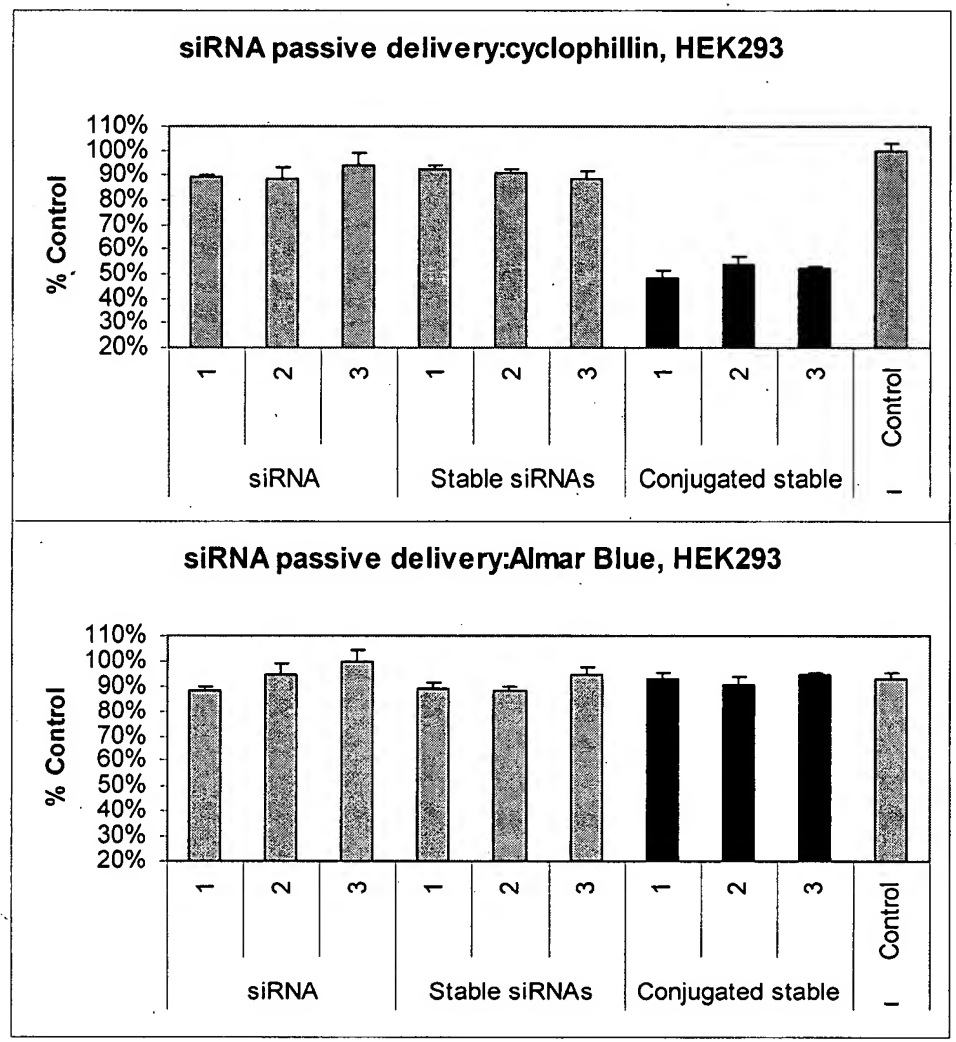



**Figure 36: The cholesterol conjugates may induce the siRNA uptake**